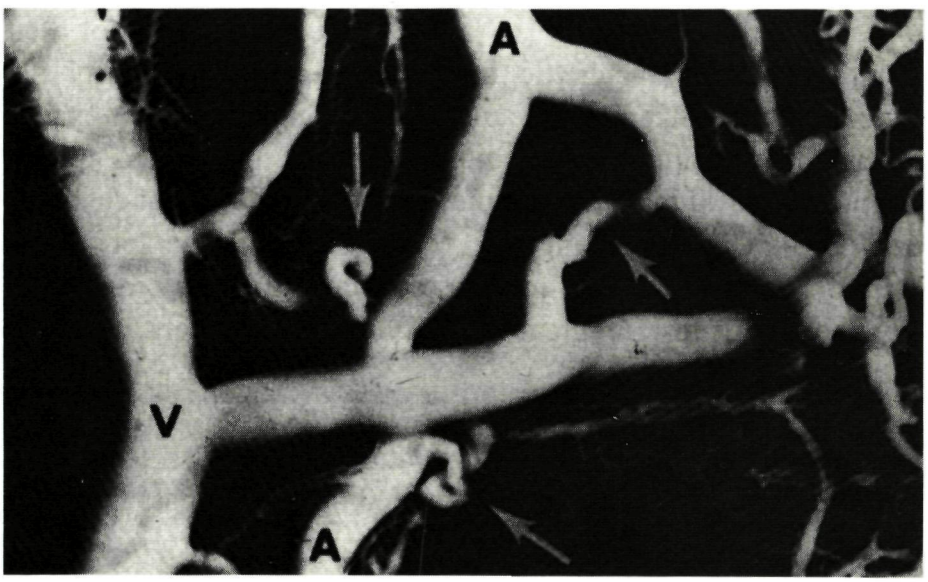
VASOSPASM AND FEMALE SEX HORMONES

STUDIES IN RAYNAUD'S PHENOMENON AND MIGRAINE



M.L. Bartelink

VASOSPASM AND FEMALE SEX HORMONES:
STUDIES IN
RAYNAUD'S PHENOMENON AND MIGRAINE

een wetenschappelijke proeve
op het gebied van de Medische Wetenschappen

PROEFSCHRIFT

ter verkrijging van de graad van doctor
aan de Katholieke Universiteit Nijmegen,
volgens besluit van het College van Decanen
in het openbaar te verdedigen
op woensdag 2 december 1992,
des namiddags te 3.30 uur precies
door

Maria Elise Ludovica Bartelink

geboren op 21 maart 1961 te Oldenzaal

Promotores: Prof. Dr Th. Thien
Prof. Dr C. van Weel
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The studies presented in this thesis were performed in the Department of Medicine, Division of General Internal Medicine and in the Department of General Practice, University of Nijmegen.

Financial support by the Netherlands Heart Foundation and the Dutch College of General Practitioners for the publication of this thesis is gratefully acknowledged. Grants from the Doctor Catharine van Tussenbroek Foundation and Organon International bv, Oss supported this publication.

Cover illustration: Skin vessels of the external ear of the horse, showing arteriovenous shunts.

CIP-GEGEVENS KONINKLIJKE BIBLIOTHEEK, DEN HAAG

Bartelink, M.L.
Vasospasm and female sex hormones: studies in Raynaud's phenomenon and migraine/ M.L. Bartelink.-[S.l.:s.n.].-Ill.
Proefschrift Nijmegen. -Met lit.opg. -Met een samenvatting in het Nederlands.
ISBN 90-9005580-0
Trefw.: vasospasme/ vrouwelijke geslachtshormonen en fenomeen van Raynaud/ vrouwelijke geslachtshormonen en migraine.

CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

In this thesis we present several epidemiological, methodological and clinical studies on vasospastic syndromes. The studies were performed in healthy volunteers, in patients with Raynaud's phenomenon (RP) and in patients with migraine.

Vasospastic syndromes

The precise pathophysiology of the mechanism which causes the characteristic attacks of white, blue and red discolorations of the fingers or toes in RP is still unknown. In primary RP arterial vasospasm, provoked by cold or emotions, is responsible for the discolorations.[1] In migraine vasospasm is also an important component of an attack. It is still a matter of debate whether this vasospasm is primarily due to vascular alterations[2] or is secondary to neural disturbances.[3] Vasospasm is also known to occur in coronary,[4] pulmonary[5] and many other arteries.[6 7 8] Moreover, the simultaneous presence of migraine or variant angina in patients with primary or secondary RP[9 10 11 12 13 14] as well as disturbances in vascular reactivity not restricted to a specific vascular bed[15 16 17] all suggest that a common systemic factor causing vasospasm might be responsible.

Epidemiology

The diagnosis RP is only rarely recorded in general practice or in population morbidity surveys, indicating that its symptoms are either not often presented to or largely underestimated by general practitioners. However, in epidemiological studies the prevalence of RP varies from 5% up to 20% in otherwise healthy females.[18 19] Reported prevalence figures of RP vary largely, depending on the different criteria used for the diagnosis, on the local climate and on the composition (for instance with regard to age and sex) of the sample group studied. Although apparently the complaints of 'dead fingers' are often not serious or alarming enough to seek medical attention, the disorder can cause great discomfort in daily life or at work, especially in the cold and wet climate of Western Europe.

Reported prevalence figures of migraine vary from 2 to 25%.[20 21 22] As in RP these differences are due to the sex and age composition of the study group, as well as to the diagnostic criteria used. Furthermore it is difficult to distinguish migraine from other types of headaches. Migraine seems to be part of a heterogeneous spectrum of disorders which forms a continuum ranging from tension headache via migraine without aura to migraine with aura.[23] Although migraine quantitatively comprises a minority of these types of headaches, its incapacitating character makes it an important disorder.

Influence of sex hormones

In primary RP the female to male ratio ranges between 2:1 and 9:1.[24 25] In studies on peripheral skin blood flow healthy women in the fertile phase of their lives show a 50% lower skin blood flow when compared with men, whereas no sex difference is observed before the menarche and after the menopause.[26 27] The severity and frequency of cold-induced vasospastic attacks[28] as well as the skin blood flow measured[29 30] vary within the menstrual cycle, although no unequivocal cyclical patterns were found. During pregnancy the frequency and the severity of the attacks were observed to diminish in some patients.[25] Moreover, anecdotal reports suggest that oral contraceptives worsen the complaints,[31 32] although paradoxically the finger blood flow has actually been observed to increase.[33]

Migraine is far more common in females than in males, particularly from the onset of puberty. A relationship between the complaints of migraine and the menstrual cycle, oral contraceptives, pregnancy and menopause has been described.[34 35]

All these findings suggest that sex hormones may influence vascular reactivity. Unfortunately, there are no studies showing a relation between physiological levels of sex hormones and the finger blood flow or the incidence or severity of migraine attacks. Moreover, the absence of unequivocal results in the above-mentioned studies makes it impossible to identify which of the sex hormones influences vasospasm and to what degree. Therefore, controversies still exist about the precise influence of sex hormones on the microcirculation and the mechanisms involved. In animal studies an influence has been found of both natural and synthetic estrogens, progestagens and androgens on the vascular reactivity of different vascular beds.[36]

The cutaneous blood flow of the fingers and the response to cold challenge is regulated by the sympathetic nervous system, which releases catecholamines that may stimulate vasoconstrictive α-adrenoceptors and vasodilating β_2-adrenoceptors. A minor disturbance anywhere within the balance between vasoconstriction and vasodilation may result in the excessive vasospasm, as already supposed by Maurice Raynaud in 1862. There are studies that indicate an influence of estrogens on the sympathetic nervous system, inducing an up-regulation of (vasoconstrictive) α_2-adrenoceptors.[37 38 39] A similar influence of the female sex hormones on vascular reactivity by modulation of the sympathetic nervous system has been claimed in patients with migraine.[40 41 42 43] In conclusion, there are a number of arguments supporting the hypothesis that sex hormones exhibit their vasoactive effects by influencing the sympathetic nervous system.

Measurement techniques and provocation tests

To objectify vasospasm the finger skin blood flow of healthy subjects and of Raynaud

patients should be measured. Quantification of peripheral skin blood flow is complicated. Under physiological conditions the skin blood flow varies extremely, even within very short periods of time. This is probably due to the pronounced sensitivity of skin blood vessels to endogenous and exogenous stimuli. Many different cold provocation tests are used to investigate vasospasm. Some apply local cold to the finger or hand, while others use contralateral or whole body cooling. Also a wide variety of measurement techniques is used, like for example the measurement of finger skin temperature,[44] laser Doppler fluxmetry,[45] finger plethysmography[46] and nailfold dynamic capillary microscopy.[47] There is little uniformity both in provocation tests and in measurement techniques. Most of the tests are not carefully standardized and their reproducibility and diagnostic values have not been stated.

In brief, in this thesis we try to find answers to the following questions:

1. What is the prevalence in males and females of both migraine and Raynaud's phenomenon? Do these disorders often occur simultaneously?
2. Do female sex hormones affect these vasospastic complaints? Do sex hormones influence the regulation of peripheral blood flow? And if so, which of the sex hormones could be responsible?

OUTLINE OF THE THESIS

The first part of this thesis evaluates epidemiological aspects of RP and migraine. To assess the influence of sex and of the female sex hormonal status on vasospastic complaints, we investigated the prevalence in males and females, and the influence of respectively menarche, menopause, pregnancy, the use of oral contraceptives and the menstrual cycle phase.

In Chapter 2 we describe the prevalence of migraine in general practice, as registered in the Continuous Morbidity Registration (CMR) project of Nijmegen[48] in accordance with the criteria of the International Classification of Health Problems in Primary Care. The combined presence of migraine and RP and the influence of the sex hormonal status on symptoms in women were investigated. A group of subjects with (non-vasospastic) tension headaches were selected from the same CMR population and were compared with the migraine group.

Chapter 3 describes the prevalence of RP in males and females, using several different criteria for its diagnosis. Furthermore, the combined presence of RP and migraine was assessed again. Since RP is not registered as a diagnosis in the CMR, a large group of patients visiting their general practitioners were investigated. The influence of their sex hormonal status on the subjective complaints of RP in patients attending the out-patient clinic are evaluated in Chapter 4.

The second part of this thesis comprises studies on the finger skin blood flow. We used a standardized finger cooling test (with local cooling in a 16°C water bath for 5 min and a recovery period of 20 min afterwards) to assess peripheral skin circulation by measurement of the finger skin temperature and the laser Doppler flux. This test has been performed in large groups of healthy subjects and in patients with RP.

First, in Chapter 5 the relation between the test results and the subjective severity of the Raynaud attacks is investigated. The reproducibility of this finger cooling test is described in Chapter 6. Its diagnostic value is evaluated and differences between males and females in test results are analyzed in Chapter 7. To assess the influence of cyclical changes and of sex hormone levels, we studied the finger skin circulation of healthy female volunteers in different phases of their menstrual cycles (Chapter 8). To improve the standardization of our provocation test (by adding a 30°C water bath before provocation to standardize baseline finger skin temperature) and to introduce a heat challenge (45°C water bath for 10 min), a combined finger heating and cooling test was used. Differences in finger skin blood flow between healthy subjects with different hormonal status were studied in four groups: males, premenopausal women, postmenopausal women and women using oral contraceptives (Chapter 9). Finally, in Chapter 10 a placebo controlled double blind study is described, in which the effect of the naturally occurring female sex hormones 17ß-estradiol and progesterone on finger skin circulation was investigated.

REFERENCES

1 Coffman JD, Davies WT. Vasospastic diseases: A review. Prog Cardiovasc Dis 1975;18:123-46.
2 Wolff HG. Headache and other headpain. New York: Oxford University Press, 1963.
3 Olesen J. The ischemic hypotheses of migraine. Neurology 1987;44:321-2.
4 Saner HE, Würbel H, Gurtner HP, Mahler F. Increased peripheral vasoconstrictor reaction upon local cold in patients with coronary heart disease. Int J Microcirc Clin Exp 1989;8:127-34.
5 Fahey PJ, Utell MJ, Goudemi JJ, Green R, Hyde RW. Raynaud's phenomenon of the lung. Am J Med 1984;76:263-9.
6 Levy A, Kuritzky A, Salomon F, Aderka D, Zoldan J, Bechar M, Pinkhas J. Raynaud's phenomenon complicated by recurrent brain infarction. Headache 1984;24:256-8.
7 Belch FF, Land D, Park RHR, McKillop JH, Mackenzie JF. Decreased oesophageal blood flow in patients with Raynaud's phenomenon. Br J Rheumatol 1988;27:426-30.
8 Mooradian AD, Viosca SP, Kaiser FE, Morley JE, Korenman SG. Penile Raynaud's phenomenon: a possible cause of erectile failure. Am J Med 1988;85:748-50.
9 Zahavi I, Chagnac A, Hering R, Davidovich S, Kuritzky A. Prevalence of Raynaud's phenomenon in patients with migraine. Arch Intern Med 1984;144:742-4.
10 Miller D, Waters DD, Warnica W, Szlachcic J, Kreeft J, Theroux P. Is variant angina the coronary manifestation of a generalized vasospastic disorder? New Engl J Med 1981;304:763-6.

11 Coffman JD, Cohen RA. Vasospasm: Ubiquitous? N Engl J Med 1981;304:780-2.
12 Atkinson AR, Appenzeller O. Hemicrania and Raynaud's phenomenon: manifestations of the same disease? Headache 1976;16:1-2.
13 Pal B, Gibson C, Passmore J, Griffiths ID, Dick WC. A study of headaches and migraine in Sjögren's syndrome and other rheumatic disorders. Ann Rheum Dis 1989;48:312-6.
14 Follansbee WP, Miller TR, Curtiss EI, Orie JE, Bernstein RL, Kiernan JM, Medsger TA. A controlled clinicopathologic study of myocardial fibrosis in systemic sclerosis (scleroderma). J Rheumatol 1990;17:656-62.
15 Gasser P, Meienberg O. Finger microcirculation in classical migraine. A video-microscopic study of nailfold capillaries. Eur Neurol 1991;31:168-71.
16 Saner HE, Würbel H, Gurtner HP, Mahler F. Increased peripheral vasoconstrictor reaction upon local cold in patients with coronary heart disease. Int J Microcirc Clin Exp 1989;8:127-34.
17 Stefenelli T, Glogar D, Dvorak I, Sochor H. Increase of pulmonary vascular resistance during cold provocation in patients with variant angina. Int J Cardiol 1988;18:27-33.
18 Olsen N, Nielsen L. Prevalence of primary Raynaud phenomena in young females. Scand J Clin Lab Invest 1978;37:761-4.
19 Maricq HR, Weinrich MC, Keil EK, Leroy EW. Prevalence of Raynaud phenomenon in the general population. J Chron Dis 1986;39:423-7.
20 Linet MS, Stewart WF. Migraine headache: Epidemiologic perspectives. Epidemiol Rev 1984;6:107-39.
21 Goldstein M, Chen TC. The epidemiology of disabling headache. In: Crithley M et al, eds. Advances in Neurology, vol 33. New York: Raven Press, 1982.
22 Bruyn GW. Epidemiology of migraine 'a personal view'. Headache 1983;23:127-33.
23 Featherstone HJ. Migraine and muscle contraction headache; a continuum. Headache 1985;25:194-8.
24 Heslop J, Coggen D, Acheson ED. The prevalence of intermittent digital ischaemia (RP) in a general practice. J Roy Coll Gen Pract 1983;33:85-9.
25 Gifford RW, Hines EA. Raynaud's disease among women and girls. Circulation 1957;16:1012-21.
26 Van de Wal HJCM, Wijn PFF, van Lier HJJ, Kneepkens WGHJ, Skotnicki SH. Noninvasive hemodynamic assessment of vasospasm in patients with primary Raynaud's phenomenon. Angiology 1987;38:315-32.
27 Bollinger A, Schlumpf M. Finger blood flow in healthy subjects of different age and sex in patients with primary Raynaud's disease. Acta Chir Scand 1975;suppl:42-7.
28 Trafford de JC, Lafferty K, Potter CE, Roberts VC, Cotton LT. An epidemiological survey of Raynaud's phenomenon. Eur J Vasc Surg 1988;2:167-70.
29 Lafferty K, de Trafford JC, Potter C, Cotton LT. Reflex vascular responses in the finger to contralateral thermal stimuli during the normal menstrual cycle: A hormonal basis to Raynaud's phenomenon? Clin Sci 1985;68:639-45.
30 Terregino CA, Seibold FACA. Influence of the menstrual cycle on Raynaud's phenomenon and on cold tolerance in normal women. Angiology 1985;36:88-95.
31 Eastcott HHG. Raynaud's disease and the contraceptive pill. Br Med J 1976;2:477.
32 Jarrett PEM. Raynaud's disease and oral contraceptives. Br Med J 1976;2:699.
33 Tooke JE, Tindall H, McNicol GP. The influence of a combined oral contraceptive pill and menstrual cycle phase on digital microvascular haemodynamics. Clin Sci 1981;61:91-5.
34 Martignom E, Sances C, Nappi G. Review: Significance of hormonal changes in migraine and cluster headache. Gynecol Endocrinol 1987;1:295-319.
35 Edelson RN. Menstrual migraine and other hormonal aspects of migraine. Headache 1985;25:376-9.

36 Altura BM, Altura BT. Influence of sex hormones, oral contraceptives and pregnancy on vascular muscle and its reactivity. In: Carrier O, Shibata S, eds. Factors influencing vascular reactivity. Tokyo: Igaku-Shoin, 1977:221-54.
37 Altura B. Sex and estrogen and responsiveness of terminal arteriole to neurohypophyseal hormones and catecholamines. J Pharmacol Exp Ther 1975;193:403-12.
38 Colucci WS, Gimbrone MA, McLaughlin MK, Halpern W, Alexander RW. Increased vascular catecholamine sensitivity and α-adrenergic receptor affinity in female and estrogen-treated male rats. Circ Res 1982;50:805-11.
39 Roberts JM, Insel PA, Goldfien RD, Goldfien A. α-Adrenoceptors but not ß-adrenoceptors increase in rabbit uterus with estrogen. Nature 1977;270:624-5.
40 Welch KMA. Migraine, a biobehavioural disorder. Neurology 1987;44:323-7.
41 Welch KMA. The role of estrogen in migraine: A review and hypothesis. Cephalalgia 1984;4:227-36.
42 Gotoh F, Komatsumoto S, Araki N, Gomi S. Noradrenergic nervous activity in migraine. Arch Neurol 1984;41:951-5.
43 Anthony M. Biochemical indices of sympathetic activity in migraine. Cephalalgia 1981;1:83-9.
44 Cleophas TJM, Fennis JFM, van 't Laar A. Finger temperature after a finger cooling test: influence of airtemperature and smoking. J Appl Physiol 1982;52:1167-71.
45 Wollersheim H, Thien Th, Fennis J, van Elteren P, van 't Laar A. Double-blind, placebo-controlled study of prazosin in Raynaud's phenomenon. Clin Pharmacol Ther 1986;40:219-25.
46 Wouda AA. Raynaud's Phenomenon. Acta Med Scand 1977;201:519-23.
47 Gasser P. Capillary blood cell velocity in finger nailfold: characteristics and reproducibility of the local cold response. Microvasc Res 1990;40:29-35.
48 Weel C van, Bosch WJHM van den, Hoogen HJM van den, Smits AJA. Development of respiratory illness in childhood - a longitudinal study in general practice. J R Coll Gen Pract 1987;37:404-8.

CHAPTER 2

MIGRAINE IN FAMILY PRACTICE: PREVALENCE AND INFLUENCE OF SEX HORMONAL STATUS

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ABSTRACT

The objective of this study was to determine the prevalence of migraine headache in a family practice patient population. In addition, this study evaluated the simultaneous presence of Raynaud's phenomenon and migraine, and examined the influence of sex hormonal status on migraine symptoms. Data from the Continuous Morbidity Registration (CMR) project of Nijmegen, the Netherlands, were used to identify all patients in the registration who had migraine headache, and to identify a comparison group of patients with non-vasospastic tension headache. Questionnaires were used to verify the diagnosis and to determine signs and symptoms of both headaches. There was an average annual prevalence of migraine headache of 4 per 1000 men and 16 per 1000 women. Eightyfive percent of patients diagnosed by family physicians as having migraine were found to fulfill the International Classification of Health Problems in Primary Care (ICHPPC) criteria for migraine headache. Migraine differed from tension headache with regard to the duration of the attacks, concomitant photo- and phonophobia, provoking factors, and the need to use analgesic medications. Raynaud's phenomenon was present in 15% of the migraine group and in 16% of the tension headache group, and occurred almost exclusively in women. The headaches were worse during and before menstruation in both groups. They were improved during pregnancy and menopause in the migraine group to greater extent than in the tension headache group. The use of the ICHPPC-criteria for migraine headache is reliable in morbidity surveys in family practice. Although there was an overlap, migraine differed in various aspects from tension headache. Digital vasospasm and an influence of female hormonal changes were present in both headache groups.

INTRODUCTION

Headache is one of the most frequently-reported health complaints.[1] Various types of headache can be discerned. Although migraine headache quantitatively comprises a minority of headaches, its incapacitating character makes it an important disorder.[2,3]

Reported prevalence of migraine varies from 2 to 25%.[2,3,4,5,6] These differences are due to various factors. First, as only 25 to 50% of all migraine patients seek professional medical care,[1,4] practice-based surveys and population surveys report different figures. Second, demographic characteristics of the population under study are important; migraine is more common in women and less-frequent at older ages.[1,2] Third, no standardized and universely accepted diagnostic criteria are used for the definition of migraine.[1,2,3,7,8] The definition formulated by the Ad Hoc Committee on the Classification of Headache was used as a standard for many years,[9] but many attempts have been made to create more specific criteria. Recently new definitions

have been formulated, including those of the International Headache Society,[10] and the criteria of the International Classification of Health Problems in Primary Care (ICHPPC).[11] Finally, migraine, with or without aura, and tension headache can be present in one patient on different occasions.[12 13] Migraine headache sometimes changes into tension headache with advancing age. It is hypothesized that migraine comprises a heterogenic spectrum of disorders, forming a continuum ranging from tension headache via migraine without aura to migraine with aura.[12 14]

The pathophysiology of migraine is controversial. Whether primary vascular alterations,[15] primary neural disturbances with secondary vascular changes,[16] dysbalance of the autonomic nervous system,[17] platelets,[18] or numerous vasoactive substances are responsible, vasospasm is an important component in the complex of a migraine attack. In fact, several studies point to a simultaneous prevalence of migraine and other vasospastic disorders, such as Raynaud's phenomenon (RP) and variant angina,[19 20 21 22 23] suggesting a common systemic vascular disturbance. Interestingly, migraine and RP are far more common in females, particularly from the onset of puberty. In addition, a relationship, though inconclusive, between migraine and the menstrual cycle,[24 25] and an influence of oral contraceptives,[26] pregnancy[27] and menopause, has been described. Therefore, it has been hypothesized that both migraine and RP may be influenced by female sex hormones.

The aim of the present study was to assess the prevalence of migraine headache in family practice according to the ICHPPC-criteria and the clinical characteristics of the patients involved. Furthermore, the combined presence of migraine and RP, and the influence of sex hormonal status on symptoms in women were investigated. A group with non-vasospastic tension headache was selected from the same population and compared with the migraine group.

METHODS

To investigate the prevalence of migraine, we used data from the Continuous Morbidity Registration project (CMR) of the Department of Family Medicine at the University of Nijmegen in the Netherlands.[28] The essential features of this registration project have recently been described.[29] It involves 12,000 persons, the practice population of the four participating practices. The registration project started in 1967, and since 1971, all four practices have participated. The number of patients has remained stable since 1971. Socio-demographic information about all patients in these practices are kept up to date. Every episode of illness seen by the family physicians or reported to them by specialists is registered and the diagnosis is classified as soon as established.

All patients of the current practice population classified as having migraine, were selected for this study. Of all patients classified, the diagnosis migraine was confirmed

with a short self-administered questionnaire, using the ICHPPC-criteria.[11] This questionnaire contained questions (Table 2.1) related to the ICHPPC-criteria for diagnosis of migraine headache. The questionnaire also included items concerning frequency and character of the headache. Inclusion in the study required either recurrent episodes of unilateral headache with at least one of the following characteristics: a) nausea or vomiting; b) aura; c) neurological disturbance, including visual; d) family history of migraine, or recurrent bilateral headaches with three or more of the above characteristics.

A group of patients with tension headache was selected from the same CMR-population. Tension headache is not a separate diagnosis in the CMR, but is classified under 'nervous disorders'. These include complaints for which no evidence of organic pathology can be found after investigation and for which a psychological explanation seems to be likely. To obtain a group not significantly different from the migraine group, two subjects were selected from this category who matched each migraine patient in sex, age, social class, and medical practice from which they received care. These patients completed the same questionnaire. Tension headache was considered present if headache occurred more than once every two months, and migraine characteristics were absent.

After this selection process was completed, a more extensive questionnaire was mailed to both the patients with migraine and the patients with tension headache. This questionnaire consisted of both precoded answers and open questions, asking about duration and perceived seriousness of the headaches, provoking factors, accompanying signs and symptoms, (self)medication, the use of professional medical services and, for women, the influence of the menstrual cycle, pregnancy, the use of oral contraceptives, and menopause. These questionnaires were personally collected at patients' home by the author (MLB), who checked for and clarified incomplete responses. Female respondents with regular menstrual cycles were asked to keep a diary during four consecutive months, recording the occurrence of headache attacks and menstrual cycles.

RP was diagnosed if the patient gave a positive answer on the following questionnaire items: complaints of cold fingers, the occurrence of a white, blue and/or red discoloration of the fingers of both hands in response to cold or emotion, and sensory disturbances during the attack and tingling afterwards.[30]

Statistical comparison of patients with migraine and patients with tension headache was performed by means of a chi-square test. P values of less than 0.05 (two-sided) were considered significant.

RESULTS

The prevalence of migraine headache among patients in the four practices was 4 per

1000 men, 16 per 1000 women, and 10 per 1000 subjects overall. During the period 1982-1987, migraine had been diagnosed in 176 patients, but 26 of these patients had left the practice by the time of the study. Thus, the questionnaire was mailed to 150 patients. There was a return response rate of 140 (93%). Frequencies of positive answers of these 140 subjects are shown in Table 2.1.

Table 2.1. Percentages of positive answers in the migraine group (n=140) on the questions in the first questionnaire. The letters a,b,c and d refer to the criteria as mentioned in the text.

Do you feel the headache at one side of the head?	88.8
Are you sick when having headache? (a)	83.7
Do you have to vomit when having headache? (a)	61.5
Do you see flashes, black spots or experience other visual disturbances preceding the headache? (b)	62.4
Do you have sensations of numbness or tickling in the face preceding the headache? (c)	34.6
Or in any other part of the body? (c)	20.2
Do you experience a weakness in a part of the body when having headache? (c)	23.8
Does anyone in your family (mother, father, sister, brother or daughter) experience the same headache? (d)	61.5

Using the ICHPPC-criteria, the prior diagnosis of migraine headache proved to be correct in 119 of the 140 patients (85%). Thirty-two patients fulfilled all four criteria for migraine, 38 fulfilled three, 35 two, and 14 one. Associated nausea or vomiting was present in all but 10 patients. There were 21 of the 140 patients who could not be classified as having migraine headache for the following reasons. In 6, the inquiry about laterality remained unanswered; in 11 the bilateral headaches did not fulfill the migraine criteria (only 4 of the 15 bilateral headaches did); and 4 patients with unilateral headache did not have another positive criterion. Age and sex characteristics of the resultant migraine group (n=119) are depicted in Figure 2.1. There was a female to male ratio of 5:1.

The questionnaire was also sent to 197 patients with nervous disorders, of whom 162 responded (82%). Fifty-nine patients had no headache at all, or had headaches less than once every two months; these 59 patients were therefore excluded. To ensure that the remaining comparison group (n=103) consisted of patients with true tension headache, subjects who might be classified as migraine based on their questionnaire responses were excluded. Thirty such subjects were excluded; 11 patients because of a family history of headache and 19 patients mostly because of headaches accompanied by nausea. After excluding these subjects tension headache

was considered to be present in 73 patients (see Figure 2.1). This group did not differ significantly from the migraine group considering practice, sex, age and social class. The diagnosis tension headache was verified by clinical interview when investigators visited subjects at home.

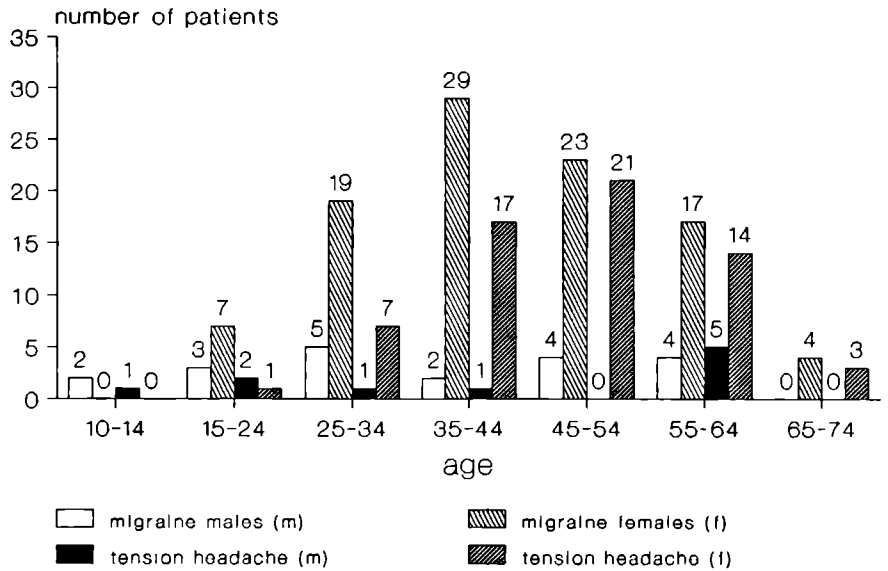

Figure 2.1. Age and sex characteristics of the respondents to the first questionnaire in the migraine group (n=119) and in the tension headache group (n=73).

The second questionnaire (see Table 2.2) was sent to all the patients with migraine according to the ICHPPC-criteria (n=119) and was completed by 102 of them (86%): 86 women and 16 men. Of the patients with tension headache, 56 responded (77% of 73): 49 women and 7 men. The remaining subjects could not be reached or refused to have the questionnaire collected by the investigator.

Photophobia and phonophobia were far more common in patients with migraine. Nearly half of the migraine group stayed at home during an attack, compared to less than 10% in the tension headache group. Frequency of the headache and character of the pain were not significantly different, but duration of the headache attack was longer in the patients with migraine. Patients with migraine used more doses of analgesic medications than did patients with tension headache. Patients with migraine also visited specialists more often, but no difference was found in frequency of visits to family physicians. An open question considering provoking factors revealed that stress and menstruation were spontaneously mentioned in both groups, whereas only patients with migraine mentioned dietary ingredients, such as coffee or alcohol.

A cyclical pattern of headache was present in 49 (57%) of the women with migraine, with headaches being more common just before or during menstruation. This cyclical occurrence of migraine was significantly more frequent ($p=0.01$) in those premenopausal women ($n=58$) who experienced premenstrual symptoms such as tenderness of breasts, weight gain, or irritability.

*Table 2.2. Clinical characteristics of the patients with migraine (n=102) and the patients with tension headache (n=56). The results are given in percentages. Significant differences (chi-square test) between the groups are represented by *.*

	Migraine		Tension headache	
Frequency				
once to several times a week	29.7		26.8	
once to several times a month	46.6		40.8	
less	23.7		32.4	
Duration				
up to hours	23.5	*	44.6	($p=0.02$)
up to one day	23.5	*	39.3	
more then one day	53.0	*	16.1	
Character				
throbbing pain (yes)	84.9		76.1	
tight feeling (yes)	53.1		56.4	
Smoking (yes)	38.2		25.0	
Staying at home (yes-always, -often)	51.0	*	9.0	($p<0.0001$)
Photophobia (yes-always, -often)	90.2	*	51.8	($p<0.0001$)
Phonophobia (yes-always, -often)	89.2	*	64.3	($p<0.0001$)
Use of medications (yes)	88.2	*	69.6	($p<0.004$)
analgesics	60.8		70.4	
ergotamin	37.3		0.0	
anti-nauseants	9.8		0.0	
Specialist consultation for the headache	19.6	*	7.3	($p<0.04$)
Family physician consultation				
once a year	35.3		25.5	
two to five times a year	57.8		70.9	
more than five times a year	6.9		3.6	
Provoking factor				
diet	31.4		0.0	
menstruation (% women)	33.7		22.4	
stress	67.6		78.6	

Among the women with tension headache, 23 (47%) reported a cyclic influence, again with an increase in frequency and seriousness of the attacks in the premenstrual period. There were no significant differences regarding this influence of the menstrual cycle between the migraine and the tension headache groups.

To investigate more thoroughly the influence of the menstrual cycle in women with migraine headache, 23 women completed prospective headache diaries. Of the total of 360 headache attacks registered in the diaries, nearly half (179) occurred in the period of 5 days before the onset of menstruation until the end of menstruation.

Questionnaire responses to the perceived effects of pregnancy, menopause, and the use of oral contraceptives are shown in Table 2.3. The influence of pregnancy on the headaches was significantly different ($p=0.01$) between patients with migraine and tension headache: the majority reported that their migraine improved and in 8 (16%) patients, headaches completely disappeared during pregnancy. Menopausal women (8 with migraine and 5 with tension headache) and postmenopausal women (28 with migraine and 22 with tension headache) were analyzed together because of small numbers and comparable results. The influence of menopause was significantly different between the both headache groups ($p=0.003$). No differences, however, were found in the effects of oral contraceptives. Subjects in both groups reported worsening of the headache, but some subjects also reported improvement and many women reported no difference in headache frequency when using oral contraceptives.

*Table 2.3. Effects of pregnancy, menopause and the use of oral contraceptives on the headache in the women with migraine and with tension headache (controls). Results are given in absolute numbers (percentages). Significant differences between the groups (chi-square test, all $p<0.01$) are represented by *.*

	Improvement	No difference	Deterioration	Do not know	Total
Pregnancy					
Migraine	32 (64)	6 (12)	7 (14)	5 (10)	50 *
Controls	6 (22)	6 (22)	0	15 (56)	27
Menopause					
Migraine	21 (58)	8 (22)	5 (14)	2 (6)	36 *
Controls	5 (19)	6 (22)	12 (44)	4 (15)	27
Oral contraceptives					
Migraine	9 (14)	24 (38)	19 (30)	12 (18)	64
Controls	1 (3)	15 (48)	5 (16)	10 (33)	31

In the group of women with migraine 8 (9%) reported the onset of the migraine headaches before the age of ten, 32 (37%) reported headache onset between the age of ten and twenty years, and 46 (54%) women had the onset of headache between twenty and fifty years of age. In only one woman, migraine started after menopause. In the group of women with tension headache, these numbers were, respectively, 4 (8%), 20 (41%), 18 (37%) and in 7 (14%) after fifty years of age. These figures differed significantly between both groups ($p=0.006$), in contrast to the figures for

males (data not shown). RP occurred in 15 (17%) of the female migraine group and in 7 (14%) of the female tension headache group (no significant difference). Two men were affected with RP, both in the tension headache group. For males and females together, the prevalence was 15% and 16%, respectively. Attacks of discoloration of the digits never occurred simultaneously with a headache. In patients who had both migraine and RP, 7 used ergotamin-containing medications during an attack and one used a ß-adrenoceptor blocking drug daily. The use of these medications did not differ significantly when compared to the total migraine group. Only one woman in the migraine group and one woman in the control group had consulted a physician for RP.

DISCUSSION

The annual prevalence of migraine headache in our population was 4 per 1000 men and 16 per 1000 women. These figures include both patients already known with migraine plus newly diagnosed cases. It is likely that in the total population, the prevalence of migraine is higher, as not all patients with migraine headache will seek medical advice.[14]

Of 140 respondents, diagnosed with migraine by their family physician, 85% fulfilled the ICHPPC-criteria. A question remains as to whether patients who did not fulfill these criteria had migraine or not. Use of a retrospective questionnaire to diagnose migraine headache might be inaccurate. In addition, the criteria used to define migraine place emphasis upon the unilaterality of the headache. In some new classifications,[10] unilaterality is not considered an obligatory feature of migraine. Among our patients who carried a diagnosis tension headache, some fulfilled criteria for migraine (unilaterality and nausea). Similarly, pulsating quality of the headache was not specific for migraine, but also occurred in patients with tension headache. These findings support the hypothesis that migraine and tension headache might form a continuum. The problems of different, sometimes non-specific, diagnostic criteria and the apparent overlap in symptoms should be kept in mind when interpreting survey data and evaluating patients.

Furthermore, this study shows that migraine, more than non-vasospastic tension headache, is influenced by female hormonal changes. The withdrawal of estrogens (as is the case premenstrually) may play a role.[31,32] In pregnancy and after the menopause, when cyclical changes in estrogen levels are absent, the migraine attacks were less frequent. But how vascular reactivity is influenced is still a matter of debate.[33,34] Some authors claim a major role for the sympathetic nervous system in migraine.[34] Estrogens have been shown to be able to increase α_2-adrenergic receptors, which have a vasoconstrictive effect after sympathetic stimulation.[35] As RP has also been associated with sympathetic nervous system imbalance,[36] the combined presence

of migraine and RP could point to a systemic sympathetic disturbance. The prevalence of RP in the population is not clearly documented and varies from 2 to 20%, according to the criteria used and the subjects studied.[37 38 39] Prevalence estimations of RP in our population range about 3% (unpublished observations). We found no higher prevalence of RP in the migraine group compared to the tension headache group. It is possible that patients with tension headache are also more liable to develop digital vasospasm in response to stress than a non-headache control group. Further studies are needed to establish whether estrogens are responsible for the influence of sex hormonal status on migraine symptoms and to establish whether sympathetic nervous system disturbances play a role.

Acknowledgements

D Panhuizen and L van Riet, medical students, assisted in collecting the data of patients with migraine. H Bor is thanked for helping in analyzing the data.

REFERENCES

1 Van de Lisdonk E. Perceived and presented morbidity in general practice. Scand J Prim Health Care 1989;7:73-8.
2 Linet MS, Stewart WF. Migraine headache: Epidemiologic perspectives. Epidemiol Rev 1984;6:107-39.
3 Goldstein M, Chen TC. The epidemiology of disabling headache. In: Crithley M et al, eds. Advances in Neurology, vol 33. New York: Raven Press, 1982.
4 Bruyn GW. Epidemiology of migraine 'a personal view'. Headache 1983;23:127-33.
5 Waters WE, O'Connor PJ. Epidemiology of headache and migraine in women. J Neurol Neurosurg Psychiat 1971;34:148-53.
6 Ziegler DK. Epidemiology of migraine. In: Clifford Rose F, ed. Handbook of Clinical neurology, vol 48. Amsterdam: Elsevier, 1986:13-22.
7 Solomon S, Guglielmo Cappa K, Smith CR. Common migraine: Criteria for diagnosis. Headache 1988;28:124-9.
8 Blau JN. Towards a definition of migraine headache. Lancet 1984;1:444.
9 Ad Hoc Committee on Classification of Headache. Classification of headache. Arch Neurol 1962;6:173-6.
10 Headache Classification Committee of the International Headache Society. Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pain. Cephalalgia 1988;8:suppl 7.
11 Classification Committee of WONCA in collaboration with the WHO. ICHPCC-2-defined (3rd edition). New York: Oxford University Press, 1983.
12 The Headache study group of the University of Western Ontario. Predictors of outcome in headache patients presenting to family physicians-a one year prospective study. Headache 1986;26:285-94.
13 Becker L, Iverson DC, Reed FM, Calonge N, Miller RS, Freeman WL. Patients with new headache in primary care: a report from ASPN. J Fam Pract 1988;27:41-7.
14 Featherstone HJ. Migraine and muscle contraction headache; a continuum. Headache 1985;25:194-8.
15 Wolff HG. Headache and other headpain. New York: Oxford University Press, 1963.

16 Olesen J. The ischemic hypotheses of migraine. Neurology 1987;44:321-2.
17 Welch KMA. Migraine, a biobehavioural disorder. Neurology 1987;44:323-7.
18 Hanington E, Jones RJ, Amess JAL, Wachowicz B. Migraine -a platelet disorder. Lancet 1981;2:720-3.
19 Zahavi I, Chagnac A, Hering R, Davidovich S, Kuritzky A. Prevalence of Raynaud's phenomenon in patients with migraine. Arch Intern Med 1984;144:742-4.
20 Miller D, Waters DD, Warnica W, Szlachcic J, Kreeft J, Theroux P. Is variant angina the coronary manifestation of a generalized vasospastic disorder? New Engl J Med 1981;304:763-6.
21 Coffman JD, Cohen RA. Vasospasm: Ubiquitous? N Engl J Med 1981;304:780-2.
22 Atkinson AR, Appenzeller O. Hemicrania and Raynaud's phenomenon: manifestations of the same disease? Headache 1976;16:1-2.
23 Gasser P, Meienberg O. Finger microcirculation in classical migraine. A video-microscopic study of nailfold capillaries. Eur Neurol 1991;31:168-71.
24 Edelson RN. Menstrual migraine and other hormonal aspects of migraine. Headache 1985;25:376-9.
25 Epstein MT, Hockaday JM, Hockaday TDR. Migraine and reproductive hormones throughout the menstrual cycle. Lancet 1975;1:543-7.
26 Dalton K. Migraine and oral contraceptives. Headache 1976;15:247-51.
27 Callaghan N. The migraine syndrome in pregnancy. Neurology 1968;18:197-201.
28 Weel C van, Bosch WJHM van den, Hoogen HJM van den, Smits AJA. Development of respiratory illness in childhood - a longitudinal study in general practice. J R Coll Gen Pract 1987;37:404-8.
29 Bogaard van den C, van den Hoogen H, Huygen FJA, van Weel C. The relationship between breast-feeding and early childhood morbidity in a general population. Fam Med 1991;23:510-5.
30 Allen E, Brown G. Raynaud's disease: a critical review of minimal requisites for diagnosis. Am J Med Sci 1932;183:187-200.
31 Somerville BW. The role of estrogen withdrawal in the etiology of menstrual migraine. Neurology 1972;22:355-65.
32 Lignieres de B, Vincens M, Mauvais-Jarvis P, Mas JL, Touboul PJ, Bousser MG. Prevention of menstrual migraine by percutaneous oestradiol. Br Med J 1986;293:1540.
33 Welch KMA. The role of estrogen in migraine: A review and hypothesis. Cephalalgia 1984;4:227-36.
34 Lundberg PO. In: Clifford Rose F, ed. Handbook of Clinical Neurology, vol 48. Amsterdam: Elsevier, 1986:431-40.
35 Roberts JM, Insel PA, Goldfien RD, Goldfien A. Alpha-adrenoceptors increase in rabbit uterus with estrogen. Nature (London) 1977;270:624-5.
36 Wollersheim H, Cleophas T, Thien Th. The role of the sympathetic nervous system in the pathophysiology and therapy of Raynaud's phenomenon. VASA 1987;Suppl 18:54-63.
37 Heslop J, Coggon D, Acheson ED. The prevalence of intermittent digital ischemia (Raynaud's phenomenon) in a general practice. J Roy Coll Gen Pract 1983;33:85-9.
38 Maricq HR, Weinrich MC, Keil JE, LeRoy EC. Prevalence of Raynaud's phenomenon in the general population. J Chron Dis 1986;39:423-7.
39 Leppert J, Åberg H, Ringqvist I, Sörensson S. Raynaud's phenomenon in a female population: Prevalence and association with other conditions. Angiology 1987;38:871-7.

CHAPTER 3

PREVALENCE OF RAYNAUD'S PHENOMENON

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Netherlands Journal of Medicine 1992;41:149-52

ABSTRACT

Studies that present prevalence rates for Raynaud's phenomenon show great variation. Figures range from a few to more than 20%. In this study, 508 patients who attended their general practitioner filled in a questionnaire dealing with symptoms of Raynaud's phenomenon. When strict criteria (cold digits, numbness and at least a biphasic discoloration) were applied, prevalence was 0.5% in males and 2.9% in females. When a monophasic white discoloration was included, prevalence figures increased by 5.4% in males and by 7.5% in females. The respective percentages became 10.4% and 21.2% when subjects with cold digits and at least a monophasic white or blue discoloration were included. Complaints of cold digits were present in 22.7% of the males and in 35% of the females. We conclude that a great deal of the variation in prevalence of Raynaud's phenomenon can be explained by differences in its definition.

INTRODUCTION

Raynaud's phenomenon (RP) is only rarely mentioned in general practice or in population morbidity surveys. This may indicate that a physician is not often confronted by a patient with these symptoms. In contrast, studies dealing with the prevalence of RP give figures ranging from a few to more than 20%.[1 2 3 4 5 6]

Considering these high figures, complaints of RP are either largely underestimated or not serious enough to seek medical attention. Indeed, only a minority of Raynaud patients consult a general practitioner and even fewer visit an out-patient clinic.[4 5]

On the other hand, RP could be an early sign of a more serious disease. Referred patients often represent the more serious cases of RP and nearly half of them can be classified as secondary RP.[7] Reliable prevalence figures of RP could provide information about the nature of this disorder: whether it is harmless and quite common in the population, or whether it indeed represents a serious warning.

Climatological differences could account for the different prevalence rates in various parts of the world. Besides geographical differences and selection of the population samples studied, differences in criteria defining RP may largely be responsible for the variation in prevalence figures. Complaints of cold fingers and feet which are associated with either mono-, bi- or triphasic discoloration and either with or without numbness are all considered by investigators to represent RP.

In the present study we assessed the prevalence of RP in a Dutch population and used several different criteria for its diagnosis.

METHODS

In three group practices, patients waiting to consult their general practitioner for any

reason, were asked at random to fill in a short questionnaire. Children under 10 yr of age and those who could not read Dutch were excluded. The questionnaire had previously been validated in a group of 44 patients with either primary or secondary RP visiting the out-patient clinic of internal medicine. Also, a group of 48 subjects known not to have complaints of RP took part in this pilot study. Sensitivity of the questionnaire was 86% and specificity 96%. The questionnaire consisted of the following yes/no items.

1. Do you regularly experience cold fingers or toes? (yes/no)
2. Do your fingers or toes regularly feel numb, so-called 'dead' fingers or toes? (yes/no)
3. Have you ever noticed your fingers or toes changing colour when they are cold? (yes/no)
   White? (yes/no) Blue? (yes/no) Red? (yes/no)
4. Have you ever consulted your general practitioner (yes/no) or a specialist (yes/no) with regard to these symptoms?

Sex and age were also filled in. The investigation took place during the period of one week in each practice in the winter. We decided that the presence of bi- or triphasic discolorations alone was not enough to diagnose RP reliably with the questionnaire. Therefore, RP was assumed if cold digits, numbness and a bi- or triphasic discoloration were present.

RESULTS

The questionnaire was completed by 508 subjects: 202 men and 306 women. The total group was divided into four age classes (10-24 yr: 29 males (6%) and 44 females (9%), 25-39 yr: 67 males (13%) and 138 females (27%), 40-54 yr: 52 males (10%) and 75 females (15%), ≥55 yr: 54 males (11%) and 49 females (10%)). Only a few patients refused to cooperate. The responses and combinations of responses to the different items are shown in Table 3.1.

Many subjects had cold digits (46 males (22.7%) and 107 females (35%)). A monophasic white (34 males (16.8%) and 54 females (17.7%)) or blue discoloration (16 males (7.9%) and 25 females (8.2%)) was also frequently present. In this study, RP was assumed if cold digits, numbness and a bi- or triphasic discoloration were present. According to these criteria, the prevalence of RP was 0.5% (1/202) in men and 2.9% (9/306) in women. This difference between men and women was significant (chi-square test, $p < 0.05$). No age-related significant difference in prevalence of RP was found. Four female patients visited a general practitioner because of their Raynaud symptoms, and one of them also consulted a specialist.

Table 3.1. Absolute and percentual (in parentheses) frequencies of the positive answers to the first three items of the questionnaire.

	Males (n=202)	Females (n=306)
Cold fingers, numbness and bi/trifasic discolorations	**1 (0.5)**	**9 (2.9)**
Cold fingers, no numbness and bi/trifasic discolorations	0	6 (2.0)
Cold fingers and bi/trifasic discolorations	1 (0.5)	15 (4.9)
Cold fingers, numbness and monofasic white discolorations	**11 (5.4)**	**23 (7.5)**
Cold fingers, no numbness and monophasic white discolorations	5 (2.5)	12 (3.9)
Cold fingers and monofasic white discolorations	16 (7.9)	35 (11.4)
Cold fingers, numbness and monophasic white or monophasic blue discolorations	**13 (6.4)**	**27 (8.8)**
Cold fingers, no numbness and monophasic white or monophasic blue discolorations	7 (3.5)	23 (7.5)
Cold fingers and monophasic white or monophasic blue discolorations	20 (9.9)	50 (16.3)
Cold fingers	46 (22.7)	107(35.0)
Numbness	50 (24.8)	72 (23.5)
Monophasic white discolorations	34 (16.8)	54 (17.7)
Monophasic blue discolorations	16 (7.9)	25 (8.2)
Bi/triphasic discolorations	10 (5.0)	23 (7.5)

If in addition to cold digits, numbness and a bi- or triphasic discoloration, a monophasic white discoloration was also included in the definition of RP, the prevalence became 5.9% in men and 10.4% in women. If a monophasic blue discoloration was included as well, the figures again increased: 6.9% (men) and 11.7% (women). When symptoms of cold digits were considered together with at least monophasic white or blue discoloration, the prevalence figures reached 10.4% (men) and 21.2% (women). Bi- or triphasic discolorations (with or without cold fingers or numbness) were present in 5.0% (men) and 7.5% (women).

DISCUSSION

Symptoms of cold extremities or a monophasic discoloration turned out to be quite common in the population. When strict criteria are applied, the prevalence of RP is much lower. Furthermore, it is shown that with less strict definitions the prevalence

figures of RP increase. Therefore, it is likely that subjects with a monophasic discoloration compose the middle of a continuum, ranging from no symptoms at one extreme and triphasic RP at the other.

In concordance with most reports,[1 4 5 6 7] in our sample more women were affected than men. Part of the sex difference may be a reflection of the female predominance in the study group. The sex difference in prevalence is, however, too convincing to attribute it completely to the distribution of males and females.

The 40- to 55-yr age group was larger than the other age categories. However, no influence of age on the questionnaire answers was found. Moreover, an influence of age in the group of RP could not be detected, although this might be due to the small numbers. It would, therefore, appear that the unequal distribution over age categories has no important effect on the results.

In the diagnosis of RP, the patient's history is the most important source of information. A questionnaire can, therefore, be used. Colour charts are also used by some investigators who try to objectify the discolorations of the digits.[6] Besides a questionnaire, a cold provocation test is used by some investigators to confirm the diagnosis of RP.[2] These tests often do not provide more diagnostic information because of the wide overlap in results between normals and patients.

In the present study we did not try to differentiate between primary and secondary RP. A questionnaire can be used to detect RP, but clinical and laboratory tests are required for further differentiation.[7]

Geographical differences can account for some of the variation in prevalence figures. Figures from warm South Carolina[5 6] could therefore be lower than those from Denmark,[2] Sweden[3] or Great Britain.[1 4] As shown by our results, differences in prevalence figures of RP can also be ascribed to differences in definitions. Until there is consensus on definitions, prevalence figures for RP are not reliable and cannot be compared.

REFERENCES

1 Silman A, Holligan S, Brennan P, Maddison P. Prevalence of symptoms of Raynaud's phenomenon in general practice. Br Med J 1990;301:590-2.

2 Olsen N, Nielsen SL. Prevalence of primary Raynaud phenomena in young females. Scand J Clin Lab Invest 1978;37:761-4.

3 Leppert J, Åberg H, Ringqvist I, Sörensson S. Raynaud's phenomenon in a female population: Prevalence and association with other conditions. Angiology 1987;38:871-7.

4 Heslop J, Coggon D, Acheson ED. The prevalence of intermittent digital ischaemia (Raynaud's phenomenon) in a general practice. J R Coll Gen Pract 1983;33:85-9.

5 Maricq HR, Weinrich MC, Keil JE, Leroy EC. Prevalence of Raynaud phenomenon in the general population. A preliminary study by questionnaire. J Chron Dis 1986;39:423-7.

6 Weinrich MC, Maricq HR, Keil JE, McGregor AR, Diat F. Prevalence of Raynaud phenomenon in the adult population of South Carolina. J Clin Epidemiol 1990;43:1343-9.

7 Wollersheim H, Thien Th. The diagnostic value of clinical signs and symptoms in patients with Raynaud's phenomenon. A cross-sectional study. Neth J Med 1990;37:171-82.

CHAPTER 4

RAYNAUD'S PHENOMENON: SUBJECTIVE INFLUENCE OF FEMALE SEX HORMONES

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ABSTRACT

Recent studies with standardized laboratory measurements of skin blood flow suggest an influence of female sex hormones on vasospasm. Therefore we evaluated the influence of the sex hormonal status on the subjective complaints of Raynaud's phenomenon and furthermore the combined presence of Raynaud's phenomenon with migraine. A detailed questionnaire was filled in by 130 primary Raynaud patients (31 males, 99 females), while 27 females, with regular menstrual cycles without the use of oral contraceptives, kept a diary with daily registration (during three months) of frequency, severity, and duration of the vasospastic attacks. Complaints improved during pregnancy in 6 out of 23 females. No influence of the menopause or the use of oral contraceptives was found. An exacerbation in some phases of the menstrual cycle was present in 15 out of 80 females. The diaries, however, did not show such influence of menstrual cycle phase. Migraine was present in 21% of the Raynaud patients. In contrast to most reports in the literature and contrary to the results of laboratory research, this study shows that most females do not experience an important subjective influence of sex hormonal status on vasospastic attacks.

INTRODUCTION

Raynaud's phenomenon (RP) is defined by attacks of an at least biphasic discoloration (white, blue or red) of fingers or toes, provoked by cold or emotional stress. Although the precise pathophysiology is unknown as yet, primary RP (PRP) is assumed to be caused by excessive arterial vasospasm.[1] Vasospasm is also known to occur in coronary,[2] pulmonary,[3] cerebral,[4] esophageal,[5] ocular[6] and penile[7] arteries. Moreover, the simultaneous presence of RP and migraine or variant angina in primary[8 9 10 11] as well as in secondary RP[12 13] suggests that this vasospasm is not restricted to the digital arteries, but that it represents a more generalized phenomenon.

RP is more common in females than in males, particularly from the onset of puberty.[1] After menopause the complaints often disappear.[1] The aggravation of RP during the use of oral contraceptives, although described in only a few patients, has led to the discussion whether RP should be regarded as a contra-indication.[14 15 16] During pregnancy symptoms were observed to improve in some patients.[17] All these findings suggest an influence of female sex hormones on the occurrence of vasospasm.

Recently the diagnostic value of the different signs and symptoms in a large group of Raynaud patients was described.[18] The present study investigates more thoroughly the influence of sex hormonal status on the complaints in women with PRP and the combined presence of PRP and migraine.

SUBJECTS AND METHODS

PRP was diagnosed in 160 subjects who visited the out-patient clinic because of complaints of digital discolorations. They all fullfilled the criteria of Allen and Brown,[19] supplemented by negative clinical signs[18] and immunological findings[20] of an underlying disease. All these 160 PRP patients (36 males and 124 females) were sent a postal questionnaire, which comprised questions with precoded answers. The questions concerned the characteristics of the Raynaud attacks, the possible co-existence of migraine and, in females, the influences of respectively menarche, the menstrual cycle, pregnancy, the use of oral contraceptives, and the menopause on the complaints. After the questionnaire had been returned, a telephone interview followed to check whether the questions were understood and the responses were filled in correctly. Hundred-thirty subjects could be contacted by telephone. Thirty subjects, of which only 11 returned the questionnaire, could not be reached, mostly because their present phonenumbers could not be traced. The remaining 130 questionnaires were analyzed.

Migraine patients were selected by means of criteria of the International Classification of Health Problems in Primary Care[21] for migraine headache. Inclusion requires either recurrent episodes of unilateral headache with at least one of the following: a) nausea or vomiting; b) aura; c) neurological disturbance, including visual; d) family history of migraine, or recurrent bilateral headaches with three or more of the above.

Physical responses to stress were investigated by the use of a standardized, validated, list with 21 yes/no questions concerning the presence of common complaints related to stress (such as palpitations, tiredness, low back pain, abdominal complaints).[22] The results of this stress list are presented as the average of the number of positive answers.

To detect a possible influence of hormonal factors, we asked for the relation between the Raynaud attacks and the phase of the menstrual cycle. It was asked whether complaints especially occurred two weeks preceding, just before, during or just after menstruation. Combinations of answers were possible. To investigate the influence of pregnancy, the use of oral contraceptives and the menopause, two questions concerning both quality and frequency of the attacks were used. A deterioration of PRP was assumed when the attacks became more severe or were more often present and improvement was assumed when the attacks became less severe or were less often present. Females were considered postmenopausal when their menstruation had been absent for more than half a year and were considered menopausal when menstruations recently became irregular with an absence of menstruation for less than half a year.

To determine the influence of the menstrual cycle on the complaints more reliably,

female Raynaud patients who had a regular menstrual cycle (between 26 and 32 days) without oral contraceptives were asked to keep a diary (n=33). This diary had been used previously to measure the subjective improvement during therapeutic studies.[23] During 3 winter months (January to March) the number of attacks, their mean severity (on a 10 point scale) and their mean duration (in min) were daily annotated. Medications were kept constant during the whole period and menstruation was registered. Afterwards, the maximum temperature of each day according to the National Weather Service and the number of the day of the menstrual cycle were added. Day -14 represented the assumed day of ovulation, while day 1 was the first day of menstruation. If a menstrual cycle did not completely fit in this 3 months period, the incomplete cycles were given cycle day numbers based upon the mean cycle length. In the analysis four cycle periods were defined: cycle day -2 to 0 (premenstrual), cycle day 1 to 3 (menstrual), cycle day -8 to -5 (mid-luteal) and the remaining cycle days (rest). Means of severity, frequency and duration and the product of all three were calculated over the four periods.

Table 4.1. Characteristics of the patients with PRP (n=130). Absolute numbers and percentages (%).

Sex	
male	31 (23.8)
female	99 (76.2)
Age (yr)	
male	42.7 ± 12.7° (range 15-72)
female	39.8 ± 11.7° (range 14-69)
Smoking	
yes	50 (38.5)
no	80 (62.5)
Medications in general	
yes	59 (45.5), 14 males, 45 females
no	71 (54.6), 17 males, 54 females
Medication for RP	
yes	66 (50.8), 17 males, 49 females
no	64 (49.2), 14 males, 50 females

°mean ± SD

RESULTS

Subjects (Table 4.1)

Questionnaires of 31 male and 99 female PRP patients were analyzed. The age of these subjects ranged between 14 and 72 yr (mean ± SD; females, 39.8 ± 11.7 and

males, 42.7 ± 12.7). About half of them used medication for RP and 38.5% were smokers.

Characteristics of the Raynaud attacks (Table 4.2)

Ninety-four percent of the patients had bilateral attacks. The frequency of the attacks differed between winter and summer. More than 40% of the patients experienced attacks only several times a month or not at all during the summer. In the winter the majority experienced attacks one or more times daily (69.8%).

Cold was mentioned by almost every subject as provoking factor, while emotions and smoking were also mentioned. Cold as the only provoking factor was present in 58.1%, cold combined with emotions in 21.7%. Emotions and smoking were never mentioned as single provoking factor. Smoking was mentioned by two subjects only. As other factors tight pressure and damp weather were mentioned.

Table 4.2. Characteristics of the attacks in patients with PRP. Absolute numbers and percentages (%).

Bilateral		
yes	118 (93.7)	
no	8 (6.3)	
missing	4	
Frequency in:	Winter	Summer
> 5/day	32 (24.8)	8 (6.3)
1-5/day	58 (45.0)	28 (21.9)
several/week	30 (23.3)	36 (28.1)
several/month	9 (7.0)	32 (25.0)
never	0	24 (18.8)
missing	1	2
Provoking factors		
cold	75 (58.1)	
cold + smoking	2 (1.6)	
cold + emotions	28 (21.7)	
cold + smoking + emotions	2 (1.6)	
cold + other	14 (10.9)	
cold + emotions + other	5 (3.9)	
do not know	2 (1.6)	
missing	2	
Family history		
yes	32°(24.6)	
no	71 (54.6)	
do not know	27 (20.8)	

°6 males and 26 females

About a quarter of the patients (24.6%, n=32), of whom 26 were females, knew of a family member with the same complaints (mother in 8, father in 8, sister in 11, brother in 5 and son and daughter in 2 cases).

Influence of sex hormonal status (Table 4.3)

Menarche

The age at which the complaints presented itself at first was not significantly different between males and females (chi-square test). However, in females the onset of complaints seems to be more common under 40 yr of age.

Pregnancy

Of the 64 women who had been pregnant, 32 women had complaints of RP before their pregnancy. An improvement of RP in pregnancy was stated by 6 of those 32 females (19%).

Menopause

Thirty-seven women were menopausal (n=6) or postmenopausal (n=31) at the time of the study of whom 24 experienced RP before menopause. In 13 females the onset of complaints was during or after the menopause. Only 4 women experienced some kind of difference (3 an improvement, 1 a deterioration) as compared with their complaints before the menopause. Most menopausal and postmenopausal women did not experience an important difference in complaints (16 experienced no difference, 4 did not know).

Oral contraceptives

Fifty-seven women used oral contraceptives. Forty females had a history of RP before they started to use the pill. Half of the women did not know whether the oral contraceptives changed their complaints, the other half had not observed any difference. Only one women experienced an exacerbation of complaints during the use of oral contraceptives.

Menstrual cycle

Eighty of the 99 females experienced complaints of RP while in their fertile period of life. In 13 females complaints started after menopause and 6 females started to use oral contraceptives soon after menarche. An influence of the menstrual cycle was only mentioned by 15 of those 80 females (19%). In this group the attacks most often occurred premenstrually.

Table 4.3. Hormonal influences on PRP. Absolute numbers and percentages (%)

Age of onset of complaints			
Yr of age	All	Males	Females
<9	7 (5.4)	1	6
10-14	31 (23.8)	4	27
15-19	11 (8.5)	1	10
20-29	30 (23.1)	7	23
30-39	27 (20.8)	8	19
40-49	15 (11.5)	7	8
>50	9 (6.9)	3	6
Influence on complaints of:	Pregnancy (n=32)	Menopause (n=24)	Oral contraceptives (n=40)
worse or more often	1	1	0
less worse or less often	6	3	1
no difference	10	16	20
do not know	15	4	19
Influence of Menstrual Cycle (n=80); exacerbation of RP:			
premenstrual			6
premenstrual and during menstruation			6
during menstruation, premenstrual and at midcycle			2
premenstrual and at midcycle			1
			(19.0)
do not know			65 (81.0)

Diaries (Table 4.4)

Twenty-seven of the initiate 33 women filled in the diary completely during these 3 months. Six diaries were filled in incompletely (because of e.g. illness) or were left out because there had been a change in medication. One diary was left out of the analysis because the product of frequency and duration regularly surmounted the 24 hr a day. First we determined the mean duration, frequency, severity of the attacks and its products in each cycle period. The mean duration of an attack was highest in the menstrual period, followed by the premenstrual period (ANOVA, $p<0.001$). Mean frequency and severity did not differ between the four cycle periods. However, some females had annotated a very long duration of their attacks and this mainly during the menstrual period. Therefore, we repeated the analysis after constructing categories for mean duration (0, 1-5, 6-15, 16-30, 31-60 min), mean severity (0, 1-4, 5-7, 8-10) and number of attacks (0, 1, 2, 3-5, >5 times daily). There were no significant differences between the cycle periods anymore.

Table 4.4. Diary results. Means ± SD of daily duration (in min), number and severity (on a 10-point scale) of the attacks and the ambient daily temperature in °C (A). Frequency, severity and duration during the whole period in percentages (B).

A		B	
duration	27.6 ± 12.4	Frequency of the attacks (%)	
number	3.1 ± 0.4	0	23.1
severity	4.3 ± 0.6	1	15.5
temperature	10.3 ± 3.9	2	15.7
		3-5	28.4
		>5	17.3
		Severity on 10-point scale (%)	
		0	23.7
		1-4	25.3
		5-7	30.0
		8-10	20.9
		Duration in minutes (%)	
		0	23.2
		1-5	17.8
		6-15	23.1
		16-30	16.2
		31-60	12.6
		>60	7.0

The outside temperature (mean, 10.3 ± 3.9°C; range, 1.2 to 21.4°C) was not significantly different in the four cycle periods. The outside temperature significantly influenced the number, the severity as well as the duration of the attacks (analysis of co-variance, $p < 0.001$).

In conclusion, with these diaries we could not find a clear influence of menstrual cycle phase on number, duration or severity of Raynaud attacks.

Migraine and stress

Headache was present in 93 patients (71.5%), whereas 37 (28.5%) patients never experienced any headache. Migraine was present in 5 males and 22 females, 21% of the PRP patients. The average number of confirmative answers on the stress list was 9.3 (out of 22).

DISCUSSION

Although RP is more often present in females, this study shows that most females do not experience an important influence of hormonal status on their complaints of RP. Migraine is frequently present, especially in the female PRP patients.

Some limitations are applicable to this study. Most patients with RP do not consult a general practitioner.[24] Even less patients are therefore seen in hospital. Consequently, this out-patient study group may not be completely representative for the Raynaud population in general. Besides, patients with RP in the out-patient department comprise a large proportion of RP secondary to an underlying disease. Although reliable screening for connective tissue disease has been carried out in our study group, it is not excluded that a number of them will eventually develop a connective tissue disease as RP can be a symptom preceding other symptoms for many years.[25 26]

Further, in contrast to a standardized measurement in the laboratory, a questionnaire deals with subjective complaints and is therefore largely depending on the attitude and recall of the subjects. No standardization of other factors, influencing the complaints of RP, is possible. It was clearly shown by the question on provoking factors and in the diaries, that cold is the most important subjective factor provoking Raynaud attacks.

General characteristics

Out of the PRP patients 38.5% were smokers. In a previous study smoking was not different between RP cases and controls.[27]

Females clearly predominate in this group of PRP patients: there were three times as much females as males. This might be a consequence of the fact that females more often consult a physician. However, a recent prevalence study carried out in the open population confirms the higher prevalence of RP in females compared with males.[27]

Hormonal influences

Under standardized laboratory conditions, modulation of finger skin blood flow reactivity by female sex hormones has been demonstrated. Studies determining finger skin blood flow confirm a lower baseline blood flow and a more pronounced vasoconstriction during cold challenge with a slower recovery afterwards in females.[28] An influence of menstrual cycle phase was demonstrated in both healthy subjects and in patients with RP.[29 30 31] Oral contraceptives possibly also influence skin blood flow.[32] Finger skin blood flow increases during pregnancy[33] as well as in postmenopausal women when compared to premenopausal women.[34] Estrogens can modulate vasoconstriction in different vessels, both in vitro and in vivo (mostly shown in animals).[35] The influence of this female sex hormone has been suggested to take place through the upregulation of vasoconstrictive α-adrenoceptors.[36] No definite prove that this hypothesis plays a role in human RP has been given yet. Moreover, although many standardized laboratory studies suggest an important influence of hormonal

factors, we were unable to detect such a clear relationship on the subjective complaints in this group of PRP patients.

However, many articles reviewing RP suggest an important influence of female sex hormones as well.[1] Most studies report that symptoms in females start in their fertile phase of life.[17 18 19 37] Other studies investigating the influence of pregnancy, the use of oral contraceptives, the menopause and the menstrual cycle, find an association with the complaints of RP.[18 37] However, in the first study[18] no questionnaire was used and in the second[37] it is unclear of which questions the used questionnaire consisted.

Maurice Raynaud already described a female patient who noticed the complete disappearance of her attacks as the first index of a commencing pregnancy. Other studies report an improvement in 45% out of 138 patients[37] and 70% out of 40 patients.[18] As pregnancy increases cardiac output and circulating blood volume, the observed increase in skin blood flow[33] may reflect these changes. The altered level of sex hormones may play an additive role. A positive influence of pregnancy is only observed in 6 of our 32 patients (19%).

The assumed negative influence of oral contraceptives[14 15 16] was not confirmed by this or other studies.[18] Therefore there seems to be no reason to discourage the use of oral contraceptives to patients with PRP.

An influence of menopause, with either improvement[1 17] or deterioration of the complaints,[37] as reported previously, was not present in our study, but this was possibly due to the small number or the short follow up.

Nineteen percent (of 32 subjects) experienced an exacerbation of symptoms during a phase of the menstrual cycle, mostly premenstrual or menstrual. This is in line with the figure of 15% out of 200 females in another study.[37] A diary study to measure the influence of the menstrual cycle has never been performed before. In this study, however, no clear influence of the cycle phase could be detected. Other factors such as outside temperature and stress may have had a stronger influence. However, only 5 of the 15 females who perceived an influence of the menstrual cycle were included in the diary group. The analysis of these 5 diaries indeed confirmed that in the mentioned phase of the cycle they experienced a deterioration of their complaints. In a way this supports the accuracy of the diaries. Perhaps a subgroup of female PRP patients experiences an influence of the menstrual cycle on their vasospasm.

Migraine

Our study confirms other studies with regard to the simultaneous presence of migraine and RP.[17 18 37 38] The vasospasm that accompanies migraine may be generalized and could be a result of an imbalance of the sympathetic nervous system, as was suggested previously.[39] Also in migraine it was suggested that estrogens may play a role in this sympathetic imbalance.[40] Therefore it could be possible that the pathophysioloy of

migraine and RP is closely related. However, an overlap in symptoms of tension headache via migraine without aura to migraine with aura exists, causing difficulties to distinguish between the various types of headache. No control group was used for this part of the questionnaire. Yet it was shown to be accurate in 85% of migraine patients in a previous study.

Stress

In almost 30% of the Raynaud patients emotional stress provoked the vasospasm, possibly by stimulating the sympathetic nervous system. To study whether Raynaud patients are more sensitive to stress, other physical complaints that might be considered as a reaction to stress were measured as well. The average sumscore on the stress list was 9.3 (of 21). In a previous study we used this stress list in a group of patients with migraine and with tension headache. The score was 6.1 for the tension headache group, a significant difference when compared to the score of 4.8 for the migraine group. The influence of stress reflects a more systemic cause, as does the combined occurrence of vasospasm elsewhere.

Conclusion

Although in many review articles on RP female sex hormones are claimed to play an important role and many standardized laboratory studies also suggest a role for sex hormones, this study shows that most females do not experience an important influence of hormonal status on their complaints. In concordance with others we found migraine to be frequently present in patients with PRP.

REFERENCES

1 Coffman JD, Davies WT. Vasospastic diseases: A review. Prog Cardiovasc Dis 1975;18:123-46.
2 Saner HE, Würbel H, Gurtner HP, Mahler F. Increased peripheral vasoconstrictor reaction upon local cold in patients with coronary heart disease. Int J Microcirc Clin Exp 1989;8:127-34.
3 Fahey PJ, Utell MJ, Goudemi JJ, Green R, Hyde RW. Raynaud's phenomenon of the lung. Am J Med 1984;76:263-9.
4 Levy A, Kuritzky A, Salamon F, Aderka D, Zoldan J, Bechar M, Pinkhas J. Raynaud's phenomenon complicated by recurrent brain infarction. Headache 1984;24:256-8.
5 Belch JJF, Land D, Park RHR, McKillop JH, Mackenzie JF. Decreased oesophageal blood flow in patients with Raynaud's phenomenon. Br J Rheumatol 1988;27:426-30.
6 Gasser P, Flammer J, Guthauser U, Mahler F. Do vasospasms provoke ocular disease? Angiology 1990;41:213-20.
7 Mooradian AD, Viosca SP, Kaiser FE, Morley JE, Korenman SG. Penile Raynaud's phenomenon: a possible cause of erectile failure. Am J Med 1988;85:748-50.

8 Zahavi I, Chagnac A, Hering R, Davidovich S, Kuritzky A. Prevalence of Raynaud's phenomenon in patients with migraine. Arch Intern Med 1984;144:742-4.
9 Miller D, Waters DD, Warnica W, Szlachcic J, Kreeft J, Theroux P. Is variant angina the coronary manifestation of a generalized vasospastic disorder? New Engl J Med 1981;304:763-6.
10 Coffman JD, Cohen RA. Vasospasm: Ubiquitous? N Engl J Med 1981;304:780-2.
11 Atkinson AR, Appenzeller O. Hemicrania and Raynaud's phenomenon: manifestations of the same disease? Headache 1976;16:1-2.
12 Pal B, Gibson C, Passmore J, Griffiths ID, Dick WC. A study of headaches and migraine in Sjögren's syndrome and other rheumatic disorders. Ann Rheum Dis 1989;48:312-6.
13 Follansbee WP, Miller TR, Curtiss EI, Orie JE, Bernstein RL, Kiernan JM, Medsger TA. A controlled clinicopathologic study of myocardial fibrosis in systemic sclerosis (scleroderma). J Rheumatol 1990;17:656-62.
14 Anonymus. Is Raynaud's disease a contraindication to a women taking the contraceptive pill? Br Med J 1976;2:156.
15 Eastcott HHG. Raynaud's disease and the oral contraceptive pill. Br Med J 1976;2:477.
16 Jarrett PEM. Raynaud's disease and oral contraceptives. Br Med J 1976;2:699.
17 Gifford RW, Hines EA. Raynaud's disease among women and girls. Circulation 1957;16:1012-21.
18 Wollersheim H, Thien Th. The diagnostic value of clinical signs and symptoms in patients with Raynaud's phenomenon. A cross-sectional study. Neth J Med 1990;37:171-82.
19 Allen EV, Brown GE. Raynaud's disease: a critical review of minimal requisites for diagnosis. Am J Med Sci 1932;183:187-200.
20 Wollersheim H, Thien Th, Hoet MH, van Venrooy WJ. The diagnostic value of several immunological tests for anti-nuclear antibody in predicting the development of connective tissue disease in patients presenting with Raynaud's phenomenon. Eur J Clin Invest 1989;19:535-41.
21 Classification Committee of WONCA in collaboration with the WHO. ICHPPC-2-defined (3rd edition). New York: Oxford University Press, 1983.
22 Dirken JM. Arbeid en stress. Thesis. Groningen: Wolters, 1969.
23 Wollersheim H, Thien Th, Fennis J, van Elteren Ph, van 't Laar A. Double-blind, placebo-controlled study of prazosin in Raynaud's phenomenon. Clin Pharmacol Ther 1986;40:219-25.
24 Heslop J, Coggon D, Acheson ED. The prevalence of intermittent digital ischaemia (Raynaud's phenomenon) in a general practice. J Roy Coll Gen Pract 1983;33:85-9.
25 Tuffanelli DL, Winkelman RK. Systemic sclerosis: a clinical study of 727 cases. Arch Dermatol 1961;84:359-71.
26 Fessel WJ. Systemic lupus erythematosis in the community. Arch Intern Med 1974;134:1017-35.
27 Weinrich MC, Maricq HR, Keil JE, McGregor AR, Diat F. Prevalence of Raynaud phenomenon in the adult population of South Carolina. J Clin Epidemiol 1990;43:1343-9.
28 Cooke JP, Creager MA, Osmundson PJ, Shephard JT. Sex differences in control of cutaneous blood flow. Circulation 1990;82:1607-15.
29 Bartelink ML, Wollersheim H, Theeuwes A, van Duren D, Thien Th. Changes in skin blood flow during the menstrual cycle: the influence of the menstrual cycle on the peripheral circulation in healthy female volunteers. Clin Sci 1990;78:527-32.
30 Lafferty K, de Trafford JC, Potter CE, Roberts VC, Cotton LT. Reflex vascular responses in the finger to contralateral thermal stimuli during the normal menstrual cycle: a hormonal basis to Raynaud's phenomenon? Clin Sci 1985;68:639-45.
31 Terregino CA, Seibold JR. Influence of the menstrual cycle on Raynaud's phenomenon and on cold tolerance in normal women. Angiology 1985;36:88-95.

32 Tooke JE, Tindall H, McNicol GP. The influence of a combined oral contraceptive pill and menstrual cycle phase on digital microvascular haemodynamics. Clin Sci 1981;61:91-5.
33 Katz M, Sokal MM. Skin perfusion in pregnancy. Am J Obstet Gynecol 1980;137:30-3.
34 Bollinger A, Schlumpf M. Finger blood flow in healthy subjects of different age and sex and in patients with primary Raynaud's disease. Acta Chir Scand 1974;suppl 465:42-7.
35 Altura BM, Altura BT. Influence of sex hormones, oral contraceptives and pregnancy on vascular muscle and its reactivity. In: Carrier O, Shibata S, eds. Factors influencing vascular reactivity. Tokyo: Igaku-Shoin, 1977;221-54.
36 Altura BM. Sex and oestrogen and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J Pharmacol Exp Ther 1975;193:403-12.
37 de Trafford JC, Lafferty K, Potter CE, Roberts VC, Cotton LT. An epidemiological survey of Raynaud's phenomenon. Eur J Vasc Surg 1988;2:167-70.
38 Leppert J, Åberg H, Ringqvist I, Sörensson S. Raynaud's phenomenon in a female population: Prevalence and association with other conditions. Angiology 1987;38:871-7.
39 Gotoh F, Komatsumoto S, Araki N, Gomi S. Noradrenergic nervous activity in migraine. Arch Neurol 1984;41:951-5.
40 Welch C. The role of estrogen in migraine: A review and hypothesis. Cephalalgia 1984;4:227-36.

CHAPTER 5

THE RELATIONSHIP BETWEEN SUBJECTIVE VASOSPASTIC COMPLAINTS AND FINGER BLOOD FLOW MEASUREMENTS IN RAYNAUD'S PHENOMENON

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ABSTRACT

The results of objective circulatory tests in patients with Raynaud's phenomenon have only rarely been related to subjective complaints. Therefore we investigated the correlation between daily self-recorded frequency, duration, and severity of vasospastic attacks and the measurement of finger skin blood flow during local cooling in 55 Raynaud patients. No significant correlations were found between complaints as registered in the diaries and, respectively, finger skin temperature and laser Doppler flux before, during, and after standardized cooling. In conclusion, finger skin blood flow measurements do not necessarily reflect digital vasospasm in daily life. Besides studies to evaluate the diagnostic value and the reproducibility of skin blood flow measurements in Raynaud's phenomenon, the relationship of test results with subjective complaints should be established.

INTRODUCTION

The diagnosis of Raynaud's phenomenon (RP) can often be made on the patient's history alone: attacks of (at least biphasic) digital discolorations, provoked by cold, or, sometimes, emotional stress. Additional cold provocation tests, with measurement of finger blood flow, are used to objectify RP and to evaluate therapy. Such tests are performed using different measurement techniques, either during local, or during whole body cooling.[1,2,3,4,5] In our clinic, a Raynaud attack is imitated by measurement of finger skin temperature (FST) and laser Doppler flux (LDF) during a standardized local cooling procedure.[1,6] Though inter- and intraindividual variation in finger skin blood flow during cold provocation is considerable, groups can reliably be compared in pathophysiological and therapeutic studies.[1,7]

The subjective severity of RP can be assessed by means of a diary in which patients daily keep record of the frequency, the duration and the severity of their attacks.[1] During therapeutic studies, a decrease in subjective complaints of patients with RP has been shown by means of this diary.[1,7]

Since other studies failed to present such a relationship, we assessed the correlation between the patient's complaints and the results of an objective finger cooling test. For this purpose, 55 Raynaud patients recorded their daily attacks in a standardized diary during 2 weeks. A finger cooling test was performed at the end of this period. Subsequently, we looked for correlations between the results of the diaries and the cooling tests.

METHODS

Subjects

We selected 55 Raynaud patients (mean age, 41.4 ± 14.1 yr); 33 patients (22 females, 11 males) with primary RP (PRP) and 22 patients (16 females, 6 males) with secondary RP (SRP). The patients with PRP fulfilled the criteria of Allen and Brown,[8] supplemented by negative clinical[9] and immunological[10] signs of an underlying disease. All SRP-patients had a connective tissue disease, fulfilling the criteria of the American Rheumatism Association (ARA),[11] or other appropriate criteria for, respectively, progressive systemic sclerosis (n=10), systemic lupus erythematosus (n=2), the CREST-syndrome (calcinosis, RP, esophageal motility disturbances, sclerodactyly, teleangiectasia; n=3), mixed connective tissue disease (n=3), rheumatoid arthritis (n=1). Three subjects had signs of a connective tissue disease but could not be classified yet according to ARA-criteria (undifferentiated connective tissue disease).

All patients had to be older than 18 years of age and should be without cardiovascular disease. They were not allowed to use vasoactive medication (if patients did use medications, these were stopped at least four weeks preceding the diary period). They experienced at least one Raynaud attack daily. All patients were normotensive (blood pressure < 160/90 mmHg).

Methods

All patients kept a diary in which they daily noted the frequency, the mean duration (in min) and the mean severity (on a 10-point scale) of the Raynaud attacks during a period of 14 days in the months of January and February. Average ambient temperature (in °C) at 4 pm, as recorded by the National Weather Service, was noted. Daily frequency, mean daily duration, mean daily severity, and its product were used in the analysis.

The day the diary period ended, a finger cooling test was performed. All tests were performed in the morning, in a quiet climatized room (ambient temperature, 24.4 ± 0.6°C; humidity, 56.9 ± 4.0%). All subjects were asked to abstain from smoking for 24 hr, from caffeine- and alcohol-containing beverages for 12 hr and to fast for at least 2 hr preceding the test.

The subjects acclimatized for at least 20 min in a comfortable supine position with their arms supported at heart level. Subsequently, the following measurements were performed. FST was measured on the second volar fingertip of the right hand (in °C; Thermocouple, Ellab Instruments, Copenhagen, Denmark). LDF was measured by an unheated probe fixed on the third volar fingertip of the right hand (in arbitrary units (AU); Periflux Pf-1d, Perimed, Stockholm, Sweden). The gain was adjusted to 3, the

cutoff frequency to 12 kHz and the time constant to 3 sec. The LDF signal was continuously written on a chart recorder. Zero calibration was performed by readjusting the pen to zero, when the probe was fixed to a white, nonmoving surface. The values were averaged during a 1-min period. The average of three pretest values obtained within five minutes were used as baseline. Subsequently, the gloved right hand was immersed to just beyond the metacarpophalangeal joints into a 16°C water bath for 5 min. Every minute momentary FST was registered. Measurement of LDF during cooling was not possible. During the recovery period of 20 min FST and LDF were obtained every 2 min.

Data Analysis

Results are given as means ± SD, unless stated otherwise. In the analysis for both FST and LDF the following parameters were used: the baseline value, the value at the end of the cooling period and the values after 12 and after 20 min of recovery.[6] Furthermore, we calculated the mean level during recovery and the mean level during the whole test by determining the area under the curve, divided by the duration of that period.[1]

Differences between patients with PRP and SRP were calculated with the Wilcoxon two sample test. As there were no significant differences between primary and secondary Raynaud patients, both in skin blood flow and in the diaries (except for the mean duration of attacks), the results of the total group of patients were used in the analysis.

Correlations between diary characteristics and the finger cooling test values were tested by the Spearman rank correlation test. A p value of less than 0.05 (two-sided) was considered to be significant.

RESULTS

Baseline FST was 29.1 ± 4.3°C. FST dropped to 18.6 ± 1.6°C after cooling and recovered up to 25.6 ± 5.0°C. The mean level during recovery was 23.4 ± 4.0°C. The mean level during the whole test was 24.3 ± 3.5°C.

Baseline LDF was 21.3 ± 17.1 AU, with a drop to 6.0 ± 6.8 AU after cooling and an increase up to 12.8 ± 15.3 AU after recovery. The mean level of LDF during recovery was 10.0 ± 11.3 AU. The daily frequency of Raynaud attacks was 4.6 ± 3.3. The mean duration of attacks was 24.2 ± 18.6 min. The mean duration in primary Raynaud patients was significantly lower than in secondary Raynaud patients (17.4 ± 11.1 versus 34.3 ± 22.9 min, $p<0.001$). Mean severity of attacks was 4.8 ± 1.8. The product of mean daily frequency, duration, and severity was 29.8 ± 39.2. The mean daily ambient temperature during the diary period was 3.0 ± 2.3°C

(minimum, -3.5°C and maximum, 6.7°C).

None of the diary characteristics showed a significant correlation with any of the finger cooling test parameters. A representive example of this poor correlation is shown in Figure 5.1 for the mean FST during the test and the product of frequency, duration, and severity of attacks. The only significant, although only weak, correlations were found between the daily ambient temperature and both FST and LDF at the end of cooling (both $r=0.3$, $p<0.05$).

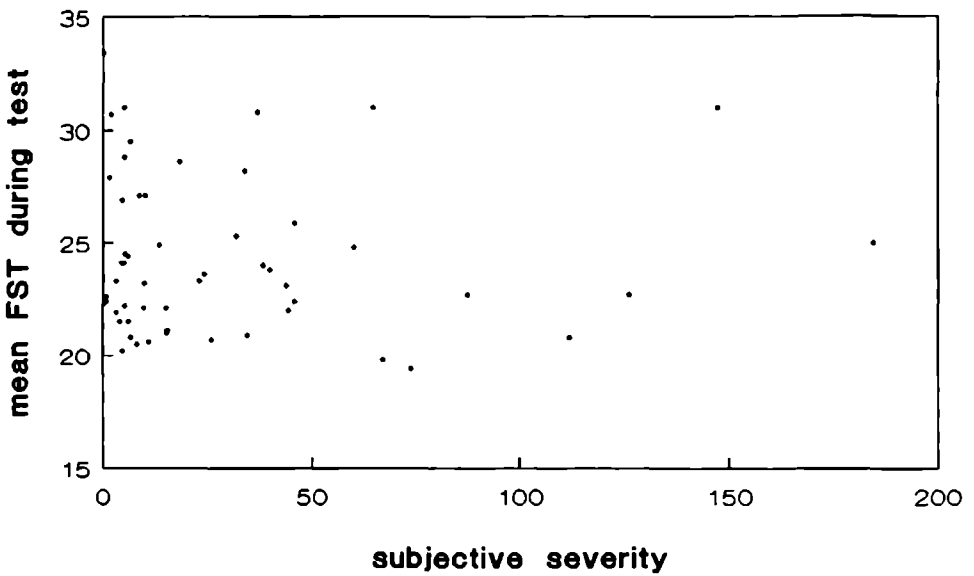

Figure 5.1. The subjective severity (expressed as the product of frequency, duration, and severity) of Raynaud attacks and the mean level of finger skin temperature (FST, in °C) during the cooling test in 55 Raynaud patients.

DISCUSSION

In this study, we found no correlation between the subjective complaints of patients with RP registered in diaries and the objective measurements of finger skin blood flow during cold challenge.

This lack of correlation can partly be explained by the fact that a cold challenge test poorly reflects the normal daily life provocation of RP, as no actual discoloration was observed in any patient. Other investigators also fail to induce the phenomenon in laboratory conditions. Failure to provoke an attack occurs with local as well as

with total body cooling, with short term as well as with long term cold exposure, and with mild as well as with extreme cold provocations.[2,3,4,5,12,13] The acclimatization period of 20 min is used to enable the subjects to relax and to exclude the influence of outside weather conditions. However, this period may be too long, or the ambient room temperature of 24.4 ± 0.6°C may be too high, preventing the development of a Raynaud attack. As stated before, total body cooling is not always a sufficient stimulus either, but we might have found a better correlation between test results and subjective complaints if local finger cooling and body cooling would have been combined. A more pronounced cold provocation might give a better correlation, but many patients experience pain and cannot sustain such low temperatures. Therefore a mild cold stimulus of a 16°C water bath was chosen.

Another factor that may contribute to the lack of correlation in this study, is the fact that the registration of subjective complaints and their severity in a diary may very well not be adequate or sufficiently standardized. This method is largely dependent on the recall and compliance of the individual patients. Other factors, besides cold, that influence the complaints, such as number of provocative moments, emotional stress, mood, and day of the menstrual cycle have not been recorded. For example, Freedman et al found that about one third of Raynaud attacks were precipitated by emotional stress, with or without accompanying cold exposure.[14] Psychological and emotional factors in daily life may play a greater role than is generally acknowledged.

In therapeutic studies we have found both subjective and objective improvement in Raynaud patients.[1,7] Tooke et al, however, noticed a considerable disparity between the results of the objective and subjective evaluation of the efficacy of ketanserin in the treatment of RP.[15] Surprisingly, we found no other studies in which the relationship between subjective complaints and objective measurements in RP is investigated. Moreover, nearly all vascular laboratories use their own exclusive cooling tests, with different measurement techniques, provocation tests, and ambient room temperatures. Also, insufficient data are available to describe the diagnostic sensitivity, specificity, or reproducibility of these tests. In conclusion, standardized laboratory measurements of finger skin blood flow appear not to reflect the multifactorial influences on Raynaud attacks in daily life. It seems mandatory that authors who publish skin blood flow data in RP present the relationship between the test results and subjective complaints, besides showing the diagnostic value and the reproducibility of their test. As long as these data are not available, current tests to evaluate RP cannot be compared nor be improved.

REFERENCES

1 Wollersheim H, Thien Th, Fennis J, van Elteren Ph, van 't Laar A. Double blind, placebo controlled study of prazosin in Raynaud's phenomenon. Clin Pharmacol Ther 1986;40:219-25.

2 Lafferty K, de Trafford JC, Roberts VC, Cotton LT. Raynaud's phenomenon and thermal entrainment: an objective test. Br Med J 1983;286:90-2.

3 Wouda AA. Raynaud's phenomenon. Photoelectric plethysmography of the fingers of persons with and without RP during cooling and warming up. Acta Med Scand 1977;201:519-23.

4 Engelhart M, Kristensen JK. Raynaud's phenomenon: blood supply to fingers during indirect cooling evaluated by laser Doppler flowmetry. Clin Physiol 1986;6:481-88.

5 Mahler F, Saner H, Boss C, Annaheim M. Local cold exposure test for capillaroscopic examination of patients with Raynaud's syndrome. Microvasc Res 1987;33:422-27.

6 Cleophas T, Fennis J, van 't Laar A. Finger skin temperature after a finger cooling test. Influence of air temperature and cigarette smoking. J Appl Physiol 1982;52:1167-171.

7 Wollersheim H, Thien Th, van 't Laar A. Nifedipine in primary Raynaud's phenomenon and in scleroderma: oral versus sublingual hemodynamic effects. J Clin Pharmacol 1987;27:907-13.

8 Allen E, Brown G. Raynaud's disease: A critical review of minimal requisites for diagnosis. Am J Med Sci 1932;183:187-200.

9 Wollersheim H, Thien Th. The diagnostical value of clinical signs and symptoms in patients with Raynaud's phenomenon. A cross-sectional study. Neth J Med 1990;37:171-82.

10 Wollersheim H, Thien Th, Hoet MH, van Venrooy WJ. The diagnostic value of several immunologic tests for antinuclear antibodies in predicting the development of connective tissue disease in patients presenting with Raynaud's phenomenon. Eur J Clin Invest 1989;19:535-41.

11 Subcommittee for scleroderma criteria of the American Rheumatism Association, Diagnostic and therapeutic criteria committee. Preliminary criteria for the classification of systemic sclerosis. Arthr Rheum 1980;23:581-90.

12 Coffman JD, Davies WT. Vasospastic diseases: a review. Prog Cardiovasc Dis 1975;18:123-46.

13 Leppert J. Limitation of finger systolic pressure measurement as a diagnostic test for primary Raynaud's phenomenon. Clin Physiol 1985;9:457-6.

14 Freedman RR, Ianni P. Role of cold and emotional stress in Raynaud's disease and scleroderma. Br Med J 1983;287:1499-502.

15 Tooke JE, Williams SA, Rawlinson DW, Black C. Ketanserin and capillary flow in Raynaud's phenomenon. Int J Microcirc Clin Exp 1990;9:249-55.

CHAPTER 6

REPRODUCIBILITY OF THE FINGER COOLING TEST

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Microvascular Research, in press

ABSTRACT

A finger cooling test is used to objectify Raynaud's phenomenon and to assess its severity. For this purpose, finger skin temperature (FST) and laser Doppler flux (LDF) are measured on the fingertips before and during cooling of the hand (16°C water bath for 5 min) and a subsequent recovery period (20 min). To study reproducibility, this test was performed twice within 3 months in 34 healthy subjects and in 56 Raynaud patients. Three test parameters were used in the analysis: the baseline value, the value after 12 min of recovery, and the mean level during recovery. We determined the limits of agreement (mean differences between the first and the second test ± 2SD) and the coefficients of variation. No systematic differences between the first and second test were found. Outside temperature did not influence FST or LDF. FST was shown to have a better reproducibility than LDF. For the baseline value in the total group the coefficient of variation was 3.3% for FST and, rather high, 21.6% for LDF. The limits of agreement for the baseline value in the total group were -4.8 to 4.2°C for FST and -25.2 to 22.2 arbitrary units for LDF. The applied cooling test has limited value in individual cases, but can be useful when comparing large groups in pathophysiologic or therapeutic studies.

INTRODUCTION

Standardized measurements of finger blood flow during cold provocation are in clinical use to objectify digital vasospasm and to differentiate healthy subjects from patients with Raynaud's phenomenon (RP).

Many different cold provocation tests are used. Some apply local cold to the finger or hand, while others use contralateral or whole body cooling. Also, a wide variety of measurement techniques is used, such as the measurement of finger skin temperature (FST),[1] laser Doppler fluxmetry (LDF),[2] finger plethysmography,[3] and nailfold dynamic capillary microscopy.[4] Because skin blood flow varies enormously under normal physiological conditions, it is important to know the reproducibility of the circulatory responses to cooling tests. Yet the response of skin blood vessels to a cold challenge during repeated application has not been studied systematically.

For years we used a standardized provocative finger cooling test with measurements of FST.[1] Later the measurement of finger skin blood flow by LDF was added.[2] In this study we report on the reproducibility of the finger cooling test by performing this test twice in a group of 34 healthy subjects and in a group of 56 Raynaud patients.

SUBJECTS AND METHODS

Subjects

We selected 34 outside controls (32 females and 2 males) and 56 Raynaud patients (40 females and 16 males). Thirty patients fulfilled the criteria of Allen and Brown,[5] and were classified as primary RP. Twenty-six patients had secondary RP due to an underlying disease. Of the secondary Raynaud patients, 8 suffered of vascular occlusive disease, revealed by angiography of the digital arteries. The remaining 18 patients had a connective tissue disease, fulfilling criteria of the American Rheumatism Association or other appropriate criteria[6]: progressive systemic sclerosis (n=8), the CREST-syndrome (calcinosis, RP, esophageal motility disturbances, sclerodactyly, teleangiectasia; n=4), polyarteriitis nodosa (n=1), systemic lupus erythematosus (n=2), rheumatoid arthritis (n=1), Sjögren's syndrome (n=1) and mixed connective tissue disease (n=1). Two had clinical signs of a connective tissue disease that could not be classified yet. Of the healthy subjects, none had any signs or symptoms of a vascular disease, none had hypertension (blood pressure >160/90 mmHg) and none used any vasoactive drug. If patients with RP used drugs for their complaints, these were stopped at least two weeks preceding a test.

Methods

In all 90 subjects two finger cooling tests were performed within a period of 3 months (mean ± SD, 21.3 ± 20.8; range, 3 to 89 days). All tests were performed in the morning, in a quiet climatized room (ambient temperature (mean ± SD), 24.5 ± 1.0°C; humidity, 59 ± 0.9%), with the subjects in a comfortable supine position with their hands supported at heart level. Outside temperature was registered as well. All subjects were asked to abstain from smoking for 24 hr and from caffeine- and alcohol-containing beverages for 12 hr and to fast for at least 2 hr preceding the test. After an acclimatization period of at least 20 min the following measurements were performed.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured at the left upper arm by an Arteriosonde (in mmHg; Roche Medical Electronics, Inc., Oranjeburg, NJ, USA).[7] Heart rate (in beats per minute) was calculated from 10 RR-intervals of an electrocardiogram strip. Forearm blood flow (FBF) was measured at the left forearm by strain gauge venous occlusion plethysmography (in ml/100 ml/min; Loosco BVP 96, Hoekloos, Amsterdam, The Netherlands). A wrist cuff was inflated up to 40-50 mmHg to exclude the venous return from the hand. FST was measured on the second volar fingertip of the right hand (in °C; Thermocouple, Ellab Instruments, Copenhagen, Denmark). LDF was measured by an unheated 90° probe

fixed on the third volar fingertip of the right hand (in arbitrary units (AU); Periflux Pf-1d, Perimed, Stockholm, Sweden). The gain was adjusted to 3, the cutoff frequency to 12 kHz and the time constant to 3 sec. The LDF signal was continuously monitored on a chart recorder. Zero calibration was performed by readjusting the pen to zero when the probe was fixed to a white, nonmoving surface. LDF values were averaged afterward on the recording as the mean during a 1-min period.

For all measurements, the average of three pretest values obtained within an interval of 10 min was used as the baseline value. Subsequently, the right hand was covered with a well-fitting plastic glove and was immersed into a water bath of 16°C for 5 min. Every minute momentary FST and mean LDF were registered. During the recovery period of 20 min FST and LDF were obtained every 2 min.

Data analysis

All calculations were performed for the whole group as well as for the subgroups of healthy subjects and Raynaud patients. Because there were no significant differences in the differences in test results between the patients with primary and secondary RP, both groups of patients with RP were joined. Results are given as means $\pm$ SD, unless stated otherwise. A p less than 0.05 (two-sided) was considered to be significant.

Baseline FBF, heart rate, SBP, and DBP were used in the analysis. The mean arterial blood pressure (MAP; in mmHg) was calculated from the formula MAP=(SBP+2DBP)/3. Differences between the healthy subjects and the Raynaud patients in these parameters were analyzed with the Student t test.

FST and LDF during the test were expressed as three separate values: the mean of three pretest values (baseline), the value after 12 min of recovery,[1] and the mean level during recovery, by determining the area under the curve during recovery divided by the duration of the recovery period.[2] The differences between the first and the second test were used to assess the reproducibility of FST and LDF.

Preliminary tests were performed to investigate the presence of possible systematic differences between the first and the second test (Wilcoxon's signed rank test).

We determined limits of agreement (the mean differences between the first and the second test $\pm$ 2SD).[8] In order to evaluate the variation of the parameters between the two tests in relation to the level of response, the coefficient of variation (in %) was also calculated for each individual subject. The average was presented as a measure of the reproducibility.

By calculating Pearson correlation coefficients, the influence of factors such as age, outside temperature, FBF, heart rate, and MAP on the differences between the test parameters was investigated as well. The influence of factors such as sex and diagnosis was considered by means of analysis of variance.

RESULTS

Some baseline characteristics of the healthy subjects and the patients are given in Table 6.1. In the group of healthy subjects females predominate. The healthy control subjects were significantly younger than the Raynaud patients ($p<0.001$). Besides age, baseline SBP, DBP, heart rate, and FBF were all significantly higher in the patients (all $p<0.01$).

Table 6.1. Characteristics of the total group (All), the healthy subjects, and the group of patients with Raynaud's phenomenon. Values are given as means ± SD, as measured in the first test.

	All	Healthy Subjects	Raynaud Patients
Number	90	34	56
Sex (male/female)	18/72	2/32	16/40
Age (yr)	34.5 ± 13.2	25.4 ± 4.2	40.0 ± 13.7
Blood pressure (mmHg)			
Systolic	117.3 ± 16.8	104.7 ± 7.7	124.7 ± 16.3
Diastolic	74.1 ± 9.8	70.0 ± 7.4	76.5 ± 10.3
Heart rate (beats/min)	69.0 ± 8.8	63.4 ± 9.1	72.5 ± 6.7
Forearm blood flow (ml/100ml/min)	3.7 ± 1.4	3.0 ± 1.6	4.1 ± 1.0

In Figure 6.1 FST and LDF during the first and the second finger cooling tests are shown for the healthy subjects and the Raynaud patients. Results of FST for the total group as well as for the healthy subjects and the Raynaud patients are shown in Table 6.2 and the corresponding values of LDF are shown in Table 6.3.

Baseline values of FST and LDF in the first and second finger cooling tests showed no significantly different results, which indicates that there were no systematic differences between the first and the second test.

Coefficients of variation for FST were lower in comparison with LDF. For all shown test parameters the limits of agreement were smaller in the Raynaud patients than in the healthy subjects. Also, the coefficient of variation was lower in the patients than in the healthy subjects. Figure 6.2 depicts the mean level during recovery of the two tests (for both FST and LDF and for both healthy subjects and Raynaud patients) in relation to the differences of these parameters between the two tests. The inter- as well as the intraindividual variability is large. The coefficient of variation, giving a relative value, best represents the increasing variability when

values of both FST and LDF are higher.

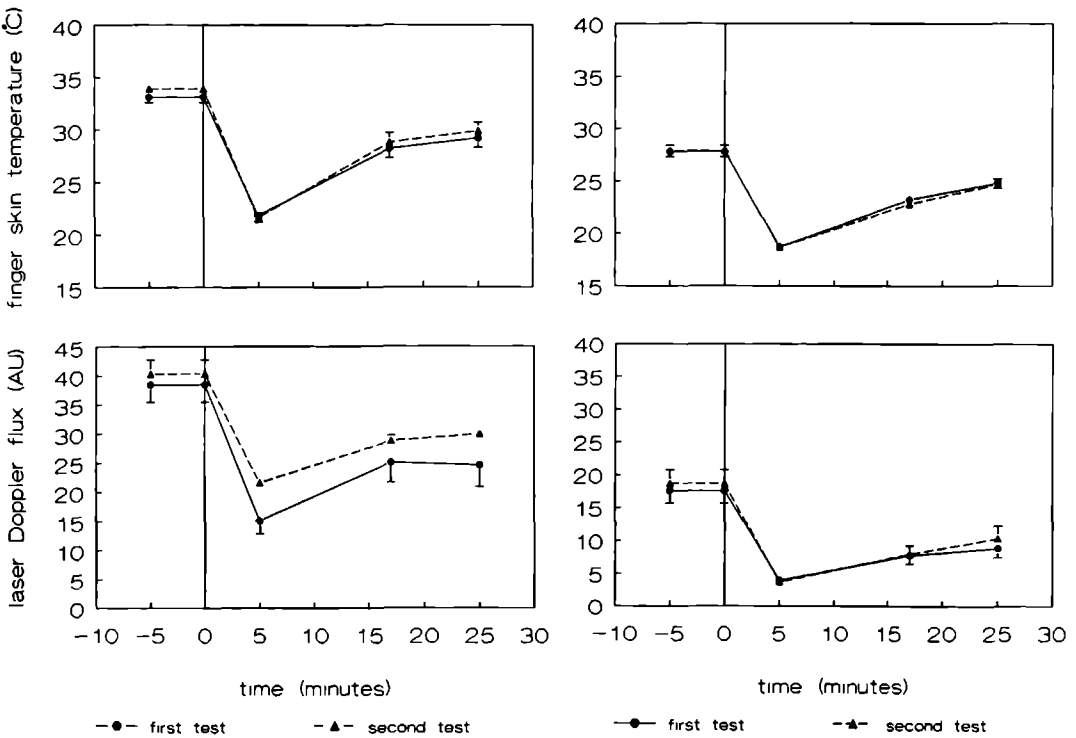

Figure 6.1. Finger skin temperature (top) and laser Doppler flux (in arbitrary units, AU, bottom) during the first and the second finger cooling tests for the healthy subjects (left) and the patients with Raynaud's phenomenon (right). Finger skin temperature and laser Doppler flux are shown before cooling (-5 to 0 min), during cooling (0 to 5 min), and during the subsequent recovery period (5 to 25 min).

There was no correlation between the mean baseline FBF of both tests (being higher in patients) and the differences of FST or LDF. Differences in baseline FBF between both tests were significantly related with differences of FST and LDF (mean level during recovery of FST, $r=0.54$, $p<0.001$; and the corresponding value of LDF, $r=0.3$, $p<0.05$).

There was no significant correlation between age, MAP, or heart rate and the differences of FST or LDF. Also, outside temperature (ranging from -4 to +32°C) did not influence reproducibility. Moreover, the difference between both finger cooling tests in MAP, heart rate, or outside temperature did not correlate with the differences in FST and LDF in the first and second tests.

Table 6.2. Reproducibility of the finger skin temperature for the total group (All, n=90), for the healthy subjects (HS, n=34), and for the patients with Raynaud's phenomenon (RP, n=56). The mean values ± SD of both tests, the limits of agreement and the coefficients of variation (CV in %) are shown.

Parameter	Group	Finger skin temp (°C) 1st test	2nd test	Limits of agreement	CV (%)
Baseline	All	29.8 ± 4.3	30.2 ± 4.5	-4.8 to 4.2	3.3
	HS	33.1 ± 2.8	33.9 ± 2.3	-6.7 to 5.1	4.0
	RP	27.8 ± 3.9	27.9 ± 3.9	-3.3 to 3.3	2.9
After 12 min recovery	All	25.1 ± 5.0	25.1 ± 4.8	-6.3 to 6.3	5.2
	HS	28.2 ± 5.3	28.8 ± 5.0	-9.4 to 8.2	8.0
	RP	23.2 ± 3.7	22.8 ± 2.9	-3.5 to 4.2	3.5
Mean level during recovery	All	24.2 ± 4.6	24.3 ± 4.3	-5.9 to 5.9	4.8
	HS	27.4 ± 4.9	27.5 ± 4.7	-8.8 to 8.4	7.9
	RP	22.4 ± 3.2	22.3 ± 2.6	-3.4 to 3.6	2.9

Table 6.3. Reproducibility of the laser Doppler flux (in arbitrary units, AU) for the total group (All, n=90), for the healthy subjects (HS, n=34), and for the patients with Raynaud's phenomenon (RP, n=56). The mean values ± SD of both tests, the limits of agreement and the coefficients of variation (CV in %) are shown.

Parameter	Group	Laser Doppler flux (AU) 1st test	2nd test	Limits of agreement	CV (%)
Baseline	All	25.4 ± 18.3	26.7 ± 18.2	-25.2 to 22.2	21.6
	HS	38.4 ± 17.4	40.3 ± 13.9	-36.4 to 32.2	26.7
	RP	17.6 ± 14.0	18.7 ± 15.5	-16.2 to 14.0	18.6
After 12 min recovery	All	14.3 ± 16.6	14.6 ± 15.2	-23.8 to 23.2	31.9
	HS	25.1 ± 20.1	25.7 ± 16.4	-35.0 to 34.4	35.4
	RP	7.8 ± 9.5	8.0 ± 9.6	-14.0 to 13.4	29.9
Mean level during recovery	All	14.5 ± 16.0	14.4 ± 14.8	-22.8 to 21.2	30.2
	HS	24.0 ± 18.7	24.0 ± 16.0	-32.1 to 31.5	34.0
	RP	7.3 ± 8.5	7.9 ± 9.6	-10.9 to 7.5	27.3

DISCUSSION

This study shows that the reproducibility of the finger cooling test is better for FST than for LDF, for all of the shown parameters.

There were no systematic differences between both tests. This implies that no adaptation to the finger cooling test was observed. Adaptation was shown before by others using finger systolic pressure.[9]

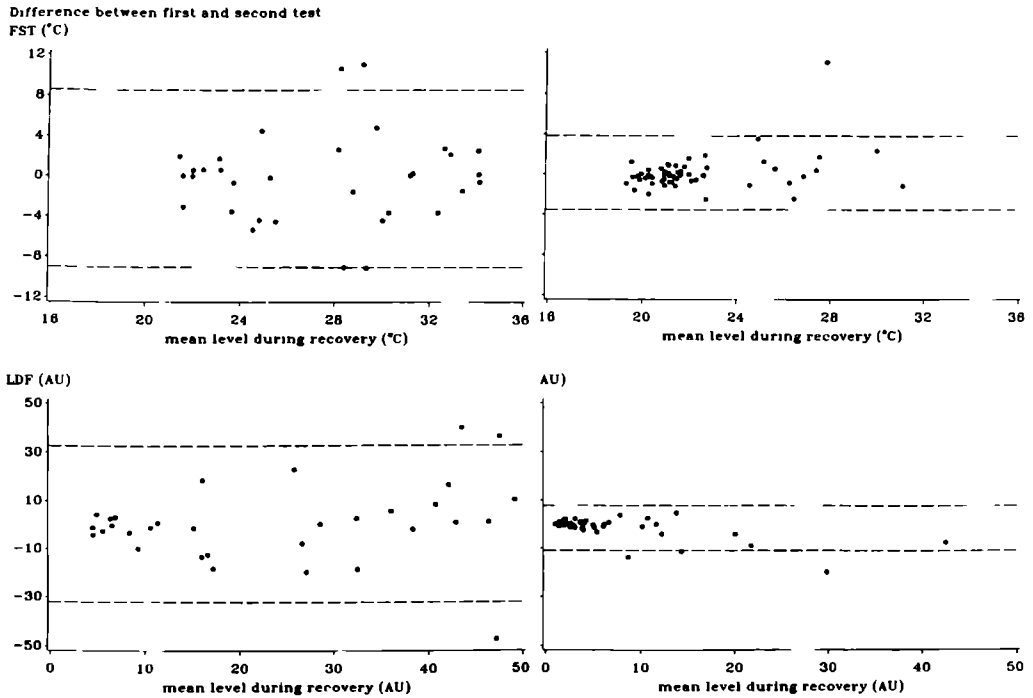

Figure 6.2. The mean level during recovery of the two tests depicted against the differences between both tests, for finger skin temperature (FST) and laser Doppler flux (LDF, in arbitrary units, AU) in the healthy subjects (left) and the patients with Raynaud's phenomenon (right). Limits of agreement are shown as well.

The large standard deviation indicates a wide interindividual variability. As can be seen by the limits of agreement and the coefficient of variation, the intraindividual variability is large as well. A great deal of this variation can be explained by biological variability. Under physiological conditions, skin blood flow varies extremely, with enormous changes within a short period. The pronounced sensitivity of skin blood vessels to endogenous and exogenous stimuli is probably responsible.

To minimize these stimuli we carefully tried to standardize several influencing factors.[1] The room temperature was constant and auditory and visual disturbances were kept to a minimum. The subjects abstained from smoking, caffeine- and alcohol-containing beverages, and food before the test. The site (always in the center of the pulp lines of the volar fingertip) and the fixation of the LDF probe (double-sided tape) were standardized. An acclimatization period of at least 20 min minimized the influence of outside temperature.

Some limitations in standardization could to some extent account for the moderate reproducibility of the test. First, individual factors such as stress are more difficult to control in a laboratory setting. Smoking habits outside the period of 24 hr could

still be of influence.

Second, in premenopausal women not using oral contraceptives the phase of the menstrual cycle is of influence[10] and tests ideally should have been performed on comparable days of the menstrual cycle.

Third, when we started to perform the LDF measurements the 25% calibration solution was not available yet. Apparatus drift might have influenced our reproducibility results slightly, although recent calibration results showed a maximal drift of 8% (mean 5%) in 3 months.

Fourth, we did not subtract the biological zero. As we found before,[11] this value is of little importance in healthy subjects, but may be important in patients with a low baseline flow. However, as it is supposed to be rather constant in one person, it is not likely to have influenced reproducibility in a major way.

Finally, biological and hemodynamic factors could influence the test results. However, as body mass index, amount of subcutaneous fat, and hand volume remain rather constant over time, reproducibility is not likely to be influenced. Blood pressure and FBF can change more substantially over time. Both could influence skin blood flow: the blood pressure by determining skin perfusion pressure and the forearm blood flow (composed mostly of muscle blood flow) possibly by a competitive mechanism with skin blood flow. The latter, however, is not substantiated by our results. As blood pressure or FBF were not significantly different between the two tests for each group, they are likely not to have influenced our results.

The better reproducibility of FST compared to LDF can partly be explained by the fact that FST is the resultant of total blood flow in the finger with a delay in reaction time. In contrast, LDF measures the blood flow instantaneously in a small volume of approximately 1 cm^3 that includes nutritional capillaries and part of the arteriovenous anastomoses.[12] LDF measurement is liable to be far more influenced by momentary changes, resulting in large temporal variation.[13,14] A slightly different site of the LDF probe in duplicate tests may influence the results as inhomogenities of the microvascular architecture in the small measuring volumes may account for the great spatial variations.[15]

The finger cooling test in healthy subjects has a worse reproducibility than in patients with RP. Some factors have to be considered.

First, Raynaud patients have a lower baseline finger skin blood flow and could therefore be less responsive to vasoconstrictive factors. Previously we have shown that vasoconstrictive reactivity is lower in the case of a low baseline skin blood flow.[16]

Furthermore, there were differences in sex between both groups. As stated before, the variable hormone levels during the female menstrual cycle could influence the peripheral circulation.[10] Although the mean age was higher in the patient group, only seven females were more than 50 years of age. The remaining females are most likely to be premenopausal and also under the influence of fluctuating hormone levels. In

the healthy subjects it was not possible to compare the reproducibility of males with females because of the relative lack of men. In the patient group no correlation was found between sex and the differences between the first and the second test. Therefore it can be assumed that sex differences between the groups do not influence reproducibility.

Finally, FBF differed significantly between the groups. To our surprise it was higher in the Raynaud patients. There was no correlation of the mean FBF and the differences in FST and LDF between both finger cooling tests, but the difference in FBF between the first and the second test (also higher in patients; data not shown) was significantly related with the differences of FST and LDF. This could indicate that the regulation of skin and muscle blood flow is somehow related.

Previously, a good reproducibility of FST in this finger cooling test was found in a small group.[1] The reproducibility of LDF is mentioned in only a few other studies and includes small subgroups only. In studies on finger skin circulation an adequate reproducibility is seldom obtained.[13 15 17 18 19] Because of the different setups of all cold provocation tests, results are difficult to compare. However, it is clear that all measurements of peripheral skin circulation are seriously hampered by the great biological variability.

It is important to know, besides reproducibility, if a test discriminates between patients and healthy subjects. We have already shown that FST discriminates well between groups of patients and healthy volunteers.[1] The finger cooling test has also been successfully used to monitor a patients' progress in several placebo-controlled therapeutic studies.[2 20]

In conclusion, the measurement of finger skin blood flow by the use of FST and LDF before, during, and after cold challenge has limited value in individual cases because of its moderate reproducibility. However, these types of tests could be useful when comparing large groups or when performing repeated measurements.

Acknowledgment

We thank J Reyenga, research nurse, for assistance in performing the finger cooling tests.

REFERENCES

1 Cleophas TJM, Fennis JFM, van 't Laar A. Finger temperature after a finger cooling test: influence of airtemperature and smoking. J Appl Physiol 1982;52:1167-71.
2 Wollersheim H, Thien Th, Fennis J, van Elteren P, van 't Laar A. Double-blind, placebo-controlled study of prazosin in Raynaud's phenomenon. Clin Pharmacol Ther 1986;40:219-25.
3 Wouda AA. Raynaud's phenomenon. Acta Med Scand 1977;201:519-23.

4 Gasser P. Capillary blood cell velocity in finger nailfold: Characteristics and reproducibility of the local cold response. Microvasc Res 1990;40:29-35.
5 Allen EV, Brown GE. Raynaud's disease, a critical review of minimal requisites for diagnosis. Am J Med Sci 1932;183:187-200.
6 Wollersheim H, Thien Th, Hoet MH, van Venrooy WJ. The diagnostic value of several immunological tests for anti-nuclear antibody in predicting the development of connective tissue disease in patients presenting with Raynaud's phenomenon. Eur J Clin Invest 1989;19:535-41.
7 Hunyor SN, Flynn JM, Cochineas C. Comparison of various sphygmomanometers with intra-arterial blood pressure readings. Br Med J 1978;2:159-62.
8 Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1986;1:307-10.
9 Wigley FM, Malamet R, Wise RA. Reproducibility of cold provocation in patients with Raynaud's phenomenon. J Rheumatol 1987;14:751-5.
10 Bartelink ML, Wollersheim H, Theeuwes A, van Duren D, Thien Th. Changes in skin blood flow during the menstrual cycle: The influence of the menstrual cycle on the peripheral circulation in healthy female volunteers. Clin Sci 1990;78:527-32.
11 Wollersheim H, Reyenga J, Thien Th. Postocclusive reactive hyperemia of fingertips, monitored by laser Doppler velocimetry in the diagnosis of Raynaud's phenomenon. Microvasc Res 1989;38:286-95.
12 Engelhart M, Kristensen JK. Evaluation of cutaneous blood flow responses by ^{133}Xenon washout and a laser Doppler flowmeter. J Invest Dermatol 1983;80:12-5.
13 Gush RJ, King TA, Jayson MIV. Aspects of laser light scattering from skin tissue with application to laser Doppler blood flow measurement. Phys Med Biol 1984;29:1463-76.
14 Stern MD, Lappe DL, Bowen PD, Chimosky JE, Holloway GA, Keiser HR, Bowman RL. Continuous measurement of tissue blood flow by laser Doppler spectroscopy. Am J Phys 1977;232:441-8.
15 Tenland T, Salerud EG, Nilsson GE, Oberg PA. Spatial and temporal variations in human skin blood flow. Int J Microcirc Clin Exp 1983;2:81-90.
16 Wollersheim H, Droste H, Reyenga J, Thien Th. Laser Doppler evaluation of skin vasomotor reflexes during sympathetic stimulation in normals and in patients with primary Raynaud's phenomenon. Int J Microcirc Clin Exp 1991;10:33-42.
17 Ninet J, Fronek A. Laser Doppler flux monitored cutaneous response to local cooling and heating. VASA 1985;14:38-43.
18 Low PA, Neumann C, Dyck PJ, Fealey RD, Tuck RR. Evaluation of skin vasomotor reflexes by using laser Doppler velocimetry. Mayo Clin Proc 1983;58:583-92.
19 Müller Ph, Keller R, Imhof P. Laser Doppler flowmetry, a reliable technique for measuring pharmacologically induced changes in cutaneous blood flow? Methods Find Clin Pharmacol 1987;9:409-20.
20 Wollersheim H, Thien Th, van 't Laar A. Nifedipine in primary Raynaud's phenomenon and in scleroderma: Oral versus sublingual hemodynamic effects. J Clin Pharmacol 1987;27:907-13.

CHAPTER 7

A STANDARDIZED FINGER COOLING TEST FOR RAYNAUD'S PHENOMENON: DIAGNOSTIC VALUE AND SEX DIFFERENCES

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European Heart Journal, in press

ABSTRACT

In the diagnosis of Raynaud's phenomenon, the patient's history is not always sufficient. Supplementary we use a standardized finger cooling test, of which the diagnostic value was investigated in the present study. In 99 patients with primary Raynaud's phenomenon, in 97 patients with secondary Raynaud's phenomenon and in 101 healthy subjects, finger skin temperature and laser Doppler flux were measured before, during and after immersion of the hand in a 16°C water bath. We determined respectively the baseline value, the values at the end of the cooling period and after 12 and 20 min of recovery, the percentual decrease during cooling and the mean levels during cooling, during recovery and during the whole test. Most of the finger skin temperature and laser Doppler flux parameters were significantly lower in females than in males in all three groups. There were no significant differences between patients with either primary or secondary Raynaud's phenomenon. In both sexes all test parameters (with exception of the percentual decrease during cooling) were significantly lower in the Raynaud patients when compared with the healthy subjects. With a diagnostic specificity of 70%, the sensitivity varied between 55% and 81% for the different test parameters. In conclusion, although in both sexes finger skin temperature and laser Doppler flux values before, during and after cooling were significantly lower in Raynaud patients compared to healthy subjects, the considerable overlap between both groups limits the diagnostic value in individual cases.

INTRODUCTION

To establish the diagnosis of Raynaud's phenomenon (RP), the patient's history is essential. RP is assumed by a history of attacks of an at least biphasic discoloration (white, blue or red) of fingers or toes, provoked by cold or emotional stress. In case the patient's history is not conclusive, an appropriate diagnostic test is needed. Such a test should also provide information regarding the severity or the prognosis of RP. Finally such a test can be used to evaluate therapy and to perform pathophysiological studies.

Clinically it is often difficult to differentiate between primary RP (PRP) and secondary RP (SRP). The latter is usually part of the clinical spectrum of a connective tissue disease. RP may precede other clinical manifestations of connective tissue diseases for many years.[1,2] Besides the patient's history and a physical examination revealing signs or symptoms of a connective tissue disease, capillary nailfold microscopy and laboratory tests for specific auto-antibodies are used to demonstrate the type of underlying connective tissue disease.[3,4]

Measurements of finger blood flow during cold provocation are widely used to differentiate healthy subjects from patients with RP and within the latter group

between the primary and the secondary subtype. Local direct and indirect cooling procedures as well as whole body cooling are used to provoke digital vasospasm. A great variety of measurement techniques is used to quantify the circulatory response.[5,6,7,8,9] No uniform, reliable and standardized test is available at present.

A finger cooling test is used in our clinic to mimic a Raynaud's attack and to assess the finger skin blood flow as measured by finger skin temperature (FST) and laser Doppler flux (LDF) before, during and after a standardized cooling procedure. Previously we have demonstrated that FST recovery after cooling has an acceptable reproducibility if room temperature and smoking are controlled.[10] Recovery was significantly slower in Raynaud patients than in healthy subjects and test results were shown to improve with successful therapeutic treatment.[11,12,13] However, since this diagnostic study was only performed in a small number of patients, larger groups of both healthy subjects and patients with either PRP or SRP were included in the present study. Test results were compared between the healthy subjects and the Raynaud patients and between the patients with PRP and with SRP.

As RP is more common in females than in males and sex differences in skin perfusion have been described in healthy subjects,[14] test results between males and females were compared within and between the different groups.

SUBJECTS AND METHODS

Subjects

We selected 99 consecutive patients with PRP (78 females, 21 males), 97 consecutive patients with SRP (66 females, 31 males) and 101 healthy subjects (66 females, 35 males). The patients with PRP fulfilled the criteria of Allen and Brown[15], supplemented with negative clinical[16] and immunological[3] signs of an underlying disease. In the group of patients with SRP 24 patients suffered from vascular occlusive disease, revealed by angiography of the digital arteries. The remaining 73 patients had a connective tissue disease, fulfilling the criteria of the American Rheumatism Association (ARA) or other appropriate criteria for respectively: progressive systemic sclerosis (n=22), systemic lupus erythematosus (n=9), the CREST-syndrome (calcinosis, RP, esophageal motility disturbances, sclerodactyly, teleangiectasia; n=6), polyarteriitis nodosa (n=2), rheumatoid arthritis (n=6), Sjögren's syndrome (n=4), mixed connective tissue disease (n=3) and dermatomyositis (n=1). Twenty subjects had signs of a connective tissue disease but could not be classified yet according to ARA criteria (undifferentiated connective tissue disease). None of the healthy subjects had any sign or symptom of a vascular disease, nor used any vasoactive drug. All were normotensive (blood pressure $<160/90$ mmHg). If patients with RP used drugs for their complaints, these were

stopped at least two weeks preceding the test.

Methods

All tests were performed in the morning, in a quiet climatized room (ambient temperature (mean ± SD), 24.4 ± 0.6°C; humidity, 56.9 ± 4.0%). All subjects were asked to abstain from smoking for 24 hr, from caffeine- and alcohol-containing beverages for 12 hr and to fast for at least 2 hr preceding the test.

The amount of subcutaneous fat (% of total body mass) was determined by measuring skinfold thickness at four standardized sites of the body (in mm), after which the corresponding percentage of subcutaneous fat according to age and sex was read from a table.[17] The hand volume (in ml) was determined by the increase in volume after immersion of the hand up to the wrist in a defined amount of water. Length (in m) and weight (in kg) were determined to calculate the body mass index.

The subjects acclimatized for at least 20 min in a comfortable supine position with their arms supported at heart level. Subsequently the following measurements were performed.

Systolic (SBP) and diastolic blood pressure (DBP) were measured at the left upper arm by an Arteriosonde (in mmHg; Roche Medical Electronics Inc., Oranjeburg, NJ, USA).[18] Heart rate (in beats per minute) was calculated from 10 RR intervals of an electrocardiogramstrip.

Forearm muscle blood flow (FBF) was measured at the left forearm by strain gauge venous occlusion plethysmography (in ml/100 ml/min; Loosco BVP 96, Hoekloos, Amsterdam, The Netherlands). A wrist cuff was inflated up to 40 mmHg to exclude the venous return from the hand.

FST was measured on the second volar fingertip of the right hand (in °C; Thermocouple, Ellab Instruments, Copenhagen, Denmark).

LDF was measured by an unheated 90° probe fixed on the third volar fingertip of the right hand (in arbitrary units (AU); Periflux Pf-1d, Perimed, Stockholm, Sweden). The gain was adjusted to 3, the cutoff frequency to 12 kHz and the time constant to 3 sec. The LDF signal was continuously written on a chart recorder. Zero calibration was performed by readjusting the pen to zero, when the probe was fixed to a white, nonmoving surface. LDF values were calculated afterwards as the mean during a 1-min period.

Transcutaneous oxygen tension (TcPO2) was measured on the fourth volar fingertip of the right hand (in mmHg; Tacomette, Novametrix Medical Systems Inc., Connecticut, USA) with the probe heated to 45°C.

In all previously mentioned measurements the average of three pretest values obtained within 10 min were used as baseline value. Subsequently, the cooling procedure followed. The right hand, with a fitting plastic glove, was immersed to

just beyond the metacarpophalangeal joints into a water bath of 16°C for 5 min. Every minute the momentary FST was registered and the mean LDF was averaged during that minute. During the recovery period of 20 min FST and LDF were obtained every 2 min. FBF, heart rate, SBP, and DBP were measured every 10 min.

Data analysis

Results are given as means ± SD, unless stated otherwise. A p value of less than 0.05 (two-tailed) was considered to be significant. While the study evolved some measurement techniques were added or changed and some results are consequently not available for the whole group.

As FBF, heart rate, SBP, or DBP did not change during the test, the baseline value was used in the analysis. The mean arterial blood pressure (MAP; in mmHg) was calculated from the formula MAP=(SBP+2DBP)/3. The body mass index was calculated by dividing weight by height^2 and expressed in kg/m^2.

In the analysis for both FST and LDF the following parameters were used: the mean of three pretest values (baseline), the value at the end of the cooling period, the percentual decrease during cooling and the value after 12 and 20 min of recovery. Furthermore, we calculated the mean level during cooling, the mean level during recovery and the mean level during the whole test by determining the area under the curve divided by the duration of that test period.[11]

Differences between groups were calculated with the Wilcoxon two sample test. Multiple (stepwise) regression was used to detect factors possibly influencing the skin circulation parameters such as blood pressure, heart rate, FBF, age, body mass index, hand volume, and amount of subcutaneous fat.

Sensitivity (i.e. the proportion of true Raynaud patients recognized) and specificity (i.e. the proportion of true negative subjects) were determined for all FST and LDF parameters during the finger cooling test, separately for males and females. We also calculated the likelihood ratio of a positive test result (sensitivity/1-specificity) and the positive predictive value (prevalence/{prevalence + (1-prevalence)/likelihood ratio}.[19]

RESULTS

General characteristics

The general characteristics of the healthy subjects and the patients with RP are shown in Table 7.1. Both groups are subdivided according to sex.

In both groups, i.e. the healthy subjects and the Raynaud patients, males were older ($p<0.05$), had a greater hand volume ($p<0.001$) and had a smaller amount of subcutaneous fat (respectively $p<0.05$ and $p<0.001$) compared with females.

Table 7.1. General characteristics of the healthy subjects and the patients with Raynaud's phenomenon (means ± SD). Both groups are divided according to sex.

	Healthy Subjects		Raynaud's Phenomenon	
	Males	Females	Males	Females
Number	35	66	52	144
Age				
(yr)	39.5 ± 13.4 @ *	34.8 ± 13.9 @	45.7 ± 12.6 *	41.2 ± 15.6
Range	21-67	17-63	14-70	12-79
Body mass index				
(kg/m^2)	23.7 ± 2.7 (28)	22.9 ± 3.4 (54)	23.2 ± 3.3 (19)	22.6 ± 4.6 (59)
Hand volume				
(ml)	485.4 ± 36.1 (12)*	334.1 ± 64.7 (53)	488.2 ± 66.8 (17)*	326.0 ± 54.1 (56)
Amount of subcutaneous fat				
(%)	25.6 ± 6.1 (13)*	30.9 ± 8.0 (53)	22.1 ± 5.8 (19)*	31.5 ± 6.0 (55)

*The numbers between parentheses refer to the number of subjects, when different from the whole group. * refers to a significant difference ($p < 0.05$) between males and females in each group. @ refers to a significant difference ($p < 0.05$) between the healthy subjects and the Raynaud patients in males and females, respectively.*

Both male and female patients were significantly older ($p<0.01$) than the male and female healthy subjects. Body mass index, the amount of subcutaneous fat and the hand volume were not significantly different between the patients and the healthy subjects.

Hemodynamic parameters

Hemodynamic parameters of the healthy subjects and of the patients with RP are shown in Table 7.2. In the healthy subjects SBP, DBP and MAP were significantly higher (all $p<0.05$) in males. In the Raynaud patients only SBP was higher in males ($p<0.05$).

In the Raynaud patients (both in the females and in the males) heart rate, SBP, DBP and MAP were significantly higher than in the healthy subjects (all $p<0.05$).

Peripheral circulation

Values of FBF and TcPO2 are shown in Table 7.2. FBF was higher in the healthy males (5.4 ± 1.4 ml/100ml/min) than in the healthy females (3.9 ± 1.6 ml/100ml/min, $p<0.05$). In the Raynaud patients there was no sex difference in FBF. FBF in the male healthy subjects (5.4 ± 1.4 ml/100ml/min) was significantly higher than in the male Raynaud patients (3.9 ± 1.2 ml/100ml/min, $p<0.01$). In females there was no difference between the Raynaud patients and healthy controls. Between the PRP and SRP patients FBF was not significantly different (data not shown).

TcPO2 did not differ between the sexes, neither in the healthy subjects nor in the Raynaud patients. TcPO2 was significantly higher in the group of healthy subjects compared with the group of patients (30.0 ± 17.4 and 22.9 ± 13.5 mmHg, $p<0.001$). In the SRP patients TcPO2 was lower when compared with the PRP patients (respectively 18.7 ± 14.5 and 27.0 ± 11.1 mmHg, $p<0.001$). When the SRP patients with scleroderma and CREST ($n=28$) were compared with the remaining patients with SRP, TcPO2 was significantly lower in the patients with scleroderma and CREST (10.3 ± 10.0 versus 22.7 ± 14.3 mmHg, $p<0.001$).

Finger cooling test

Finger skin temperature

All FST parameters were significantly higher in the control subjects than in the Raynaud patients (all $p<0.001$). Between the PRP and SRP patients no significant differences in FST parameters were found. When both groups of healthy subjects and Raynaud patients were divided according to sex (see Figure 7.1 (upper panel) and Table 7.3 (upper panel)), the following differences were observed. Healthy females,

when compared with healthy males, recovered more slowly after cooling: the mean level during recovery for females was 27.4 ± 4.8°C and for males 30.7 ± 3.9°C, $p<0.01$. Female Raynaud patients, when compared with male patients, had a lower baseline FST (females, 28.6 ± 4.1°C; males, 31.3 ± 3.8°C, $p<0.001$) and they also recovered more slowly after cooling (mean level during recovery in females, 23.1 ± 3.8°C; males, 26.6 ± 4.8°C, $p<0.001$).

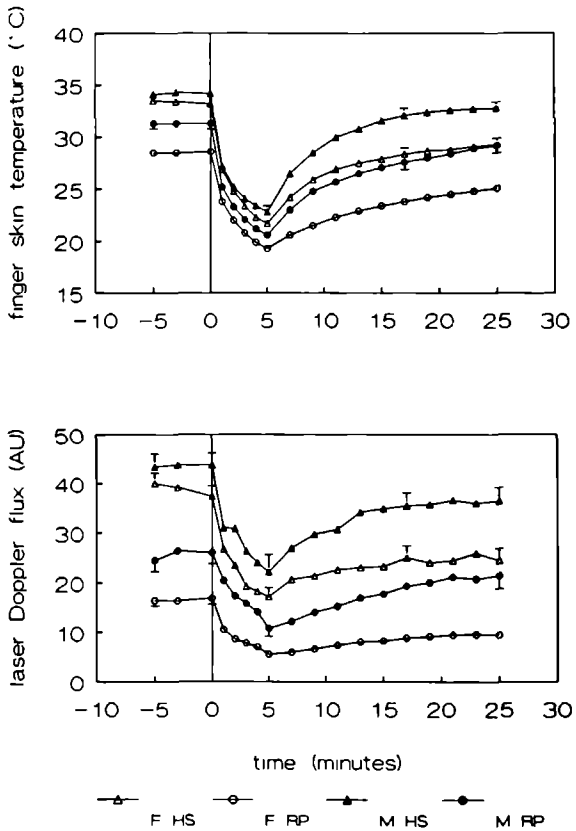

Figure 7.1. Finger skin temperature (upper panel) and laser Doppler flux, in arbitrary units (AU, lower panel), means ± SEM before and during cooling in a 16°C water bath and during 20 min of recovery afterwards in the female healthy subjects (F HS), the male healthy subjects (M HS), the female patients with Raynaud's phenomenon (F RP) and the male patients with Raynaud's phenomenon (M RP).

When comparing the male healthy subjects with the male patients, all FST parameters except for the percentual decrease during cooling were significantly higher in the male healthy subjects (all $p<0.001$). In the females all FST parameters were significantly higher in the healthy subjects than in the Raynaud patients (all $p<0.001$).

Table 7.2. Hemodynamic baseline parameters in the healthy subjects and the patients with Raynaud's phenomenon (means ± SD). Both groups are divided according to sex.

	Healthy Subjects		Raynaud's Phenomenon	
	Males	Females	Males	Females
Number	35	66	52	144
Blood pressure (mmHg)				
Systolic	114.0 ± 10.2 @ *	107.1 ± 9.6 @	125.7 ± 16.3 *	119.5 ± 19.0
Diastolic	73.2 ± 6.7 @ *	70.2 ± 6.1 @	78.3 ± 10.1	76.4 ± 10.0
Mean	86.8 ± 6.8 @ *	82.5 ± 6.3 @	94.1 ± 11.3	90.8 ± 11.9
Heart rate (beats/min)	66.4 ± 9.2 @	66.3 ± 9.7 @	71.6 ± 9.7	72.3 ± 9.4
Forearm blood flow (ml/100ml/min)	5.4 ± 1.4 @ (9) *	3.9 ± 1.6 (43)	3.9 ± 1.2 (43)	3.6 ± 1.6 (130)
Transcutaneous oxygen pressure (mmHg)	40.0 ± 15.2 @ (25)	37.3 ± 11.3 @ (47)	24.6 ± 14.6	22.8 ± 12.8

*The numbers between parentheses refer to the number of subjects, when different from the whole group. * refers to a significant difference ($p < 0.05$) between males and females in each group. @ refers to a significant difference ($p < 0.05$) between the healthy subjects and the Raynaud patients in males and females, respectively.*

In Table 7.4 (upper panel) the FST data between the PRP patients and the SRP patients are compared, again divided according to sex. In the PRP patients all parameters were significantly lower in females compared with males (all $p<0.001$). Also in the SRP patients these differences were present (all $p<0.05$), except for respectively the baseline value, the percentual decrease during cooling and the mean level during cooling.

When comparing either the male PRP patients with the male SRP patients or the female PRP patients with the female SRP patients, no significant differences were observed.

Laser Doppler flux

All LDF parameters, with the exception of the percentual decrease during cooling, were significantly higher in the healthy subjects (all $p<0.001$). Between the PRP and SRP patients no significant differences in the LDF parameters were seen.

In Figure 7.1 (lower panel) and Table 7.3 (lower panel) the LDF values during the finger cooling test are shown separately for the males and for the females in the healthy subjects as well as in the Raynaud patients.

In accordance with FST, LDF was significantly different between males and females in both groups. When compared with healthy males, healthy females recovered more slowly after cooling: the mean level during recovery for females was 23.4 ± 17.4 AU and for males 32.5 ± 16.3 AU, $p<0.01$. In the patients with RP, women had a lower baseline value (females, 16.6 ± 13.4 AU; males, 25.7 ± 15.5 AU, $p<0.001$) and they also recovered more slowly after cooling than males (mean level during recovery in females, 8.2 ± 9.0 AU; males, 17.5 ± 15.4 AU, $p<0.001$). In Table 7.4 (lower panel) all LDF parameters of the PRP patients and the SRP patients are compared for both males and females. In the PRP patients all parameters were significantly lower in females compared with males (all $p<0.001$). In the SRP patients these significant differences were also present (all $p<0.05$), except for the baseline value, the percentual decrease during cooling and the mean level during cooling.

When comparing either the male PRP patients with the male SRP patients or the female PRP patients with the female SRP patients no significant differences in LDF parameters were observed. Finally, the patients with scleroderma or CREST, when compared with the remaining SRP patients, showed a significantly lower baseline LDF, LDF after cooling, and after 12 and 20 min of recovery, and mean LDF during recovery (all $p<0.05$, data not shown).

Table 7.3. Means ± SD of finger skin temperature parameters (FST, upper panel) and laser Doppler flux parameters (LDF, in arbitrary units (AU), lower panel) before, during and after cooling in the healthy subjects and in the patients with Raynaud's phenomenon. Both groups are divided according to sex (M=males and F=females). The numbers between parentheses refer to the number of subjects, when different from the whole group.

	Healthy Subjects				Raynaud's Phenomenon)			
	M (n=35)		F (n=66)		M (n=52)		F (n=144)	
FST (°C)								
Baseline	34.2 ± 1.3 @		33.4 ± 2.5 @		31.3 ± 3.8	*	28.6 ± 4.1	
End of cooling	22.8 ± 3.4 @		21.7 ± 3.0 @		20.6 ± 2.5	*	19.3 ± 2.1	
Decrease (%)	33.5 ± 8.8		35.1 ± 7.8 @		33.7 ± 7.4		31.9 ± 6.6	
Mean level during cooling	25.0 ± 2.2 @		24.3 ± 2.2 @		22.9 ± 2.2	*	21.6 ± 2.2	
After 12 min of recovery	32.1 ± 3.9 @	*	28.4 ± 5.0 @		27.6 ± 5.2	*	23.8 ± 4.2	
End of recovery	32.8 ± 3.3 @	*	29.3 ± 4.8 @		29.2 ± 5.0	*	25.1 ± 4.3	
Mean level during recovery	30.7 ± 3.9 @	*	27.4 ± 4.8 @		26.6 ± 4.8	*	23.1 ± 3.8	
Mean level during test	30.6 ± 3.0 @	*	28.3 ± 3.6 @		27.1 ± 3.9	*	24.0 ± 3.4	
LDF (AU)								
Baseline	43.6 ± 15.4 @		38.9 ± 16.6 @		25.7 ± 15.4	*	16.6 ± 13.4	
End of cooling	22.2 ± 19.8 @		17.2 ± 15.1 @		10.8 ± 10.8	*	5.6 ± 6.3	
Decrease (%)	50.7 ± 32.8		57.3 ± 28.5		52.4 ± 45.5		59.3 ± 35.7	
Mean level during cooling	27.0 ± 16.3 @ (28)		22.0 ± 14.7 @ (58)		17.0 ± 13.6 (22)	*	8.5 ± 7.7 (61)	
After 12 min of recovery	35.5 ± 16.1 @	*	25.1 ± 19.6 @		19.4 ± 19.0	*	8.9 ± 10.1	
End of recovery	36.5 ± 16.0 @	*	24.6 ± 18.3 @		21.5 ± 18.5	*	9.5 ± 11.0	
Mean level during recovery	32.5 ± 16.3 @	*	23.4 ± 17.4 @		17.5 ± 15.4	*	8.2 ± 9.0	
Mean level during test	33.2 ± 15.8 @ (28)		26.4 ± 15.7 @ (58)		19.4 ± 15.8 (22)	*	11.2 ± 9.0 (61)	

** Refers to a significant difference ($p<0.05$) between males and females in each group.*

@ Refers to a significant difference ($p<0.05$) between the male healthy subjects and the male Raynaud patients, respectively between the female healthy subjects and the female Raynaud patients.

Table 7.4. Means ± SD of finger skin temperature parameters (FST, upper panel) and laser Doppler flux parameters (LDF, in arbitrary units (AU), lower panel) before, during and after cooling in the patients with primary (PRP) and with secondary (SRP) Raynaud's phenomenon. Both groups are divided according to sex (M=males and F=females). The numbers between parentheses refer to the number of subjects, when different from the whole group.

	PRP				SRP			
	M (n=21)		F (n=78)		M (n=31)		F (n=66)	
FST (°C)								
Baseline	32.4 ± 2.9	*	28.1 ± 4.3		30.5 ± 4.1		29.1 ± 3.9	
End of cooling	20.7 ± 2.4	*	19.1 ± 1.9		20.6 ± 2.6	*	19.4 ± 2.2	
Decrease (%)	36.0 ± 7.1	*	31.3 ± 6.6		32.1 ± 7.3		32.6 ± 6.4	
Mean level during cooling	23.4 ± 1.8	*	21.5 ± 2.3		22.6 ± 2.4		21.8 ± 2.2	
After 12 min of recovery	28.1 ± 5.0	*	23.5 ± 4.1		27.2 ± 5.4	*	24.1 ± 4.4	
End of recovery	30.2 ± 4.5	*	24.7 ± 4.2		28.5 ± 5.2	*	25.5 ± 4.5	
Mean level during recovery	27.2 ± 4.5	*	26.2 ± 5.0		26.2 ± 5.0	*	23.5 ± 4.1	
Mean level during test	27.7 ± 3.5	*	23.7 ± 3.3		26.6 ± 4.2	*	24.3 ± 3.4	
LDF (AU)								
Baseline	28.3 ± 14.5	*	17.8 ± 14.3		23.8 ± 16.0	*	15.2 ± 12.2	
End of cooling	9.9 ± 8.6	*	5.7 ± 6.5		11.5 ± 12.2	*	5.6 ± 6.1	
Decrease (%)	63.8 ± 23.5		60.8 ± 37.6		45.1 ± 54.4		57.6 ± 33.4	
Mean level during cooling	10.0 ± 7.4 (10)		9.8 ± 8.3 (37)		22.8 ± 15.0 (12)	*	6.7 ± 6.4 (24)	
After 12 min of recovery	20.4 ± 16.6	*	8.5 ± 9.4		18.7 ± 20.8	*	9.3 ± 11.0	
End of recovery	21.8 ± 15.1	*	9.2 ± 10.7		21.3 ± 20.7	*	9.8 ± 11.4	
Mean level during recovery	18.4 ± 13.4	*	8.0 ± 8.2		16.9 ± 16.8	*	8.5 ± 9.8	
Mean level during test	14.7 ± 11.8 (10)		12.8 ± 9.3 (37)		23.4 ± 18.0 (12)	*	8.8 ± 8.0 (24)	

** Refers to a significant difference ($p<0.05$) between males and females in each group.*

Influencing factors

No significant influence of hemodynamic (MAP, heart rate and FBF) and of biological factors (age, body mass index, amount of subcutaneous fat and hand volume) on the different test parameters was found when tested by multiple regression.

Discriminating diagnostic parameters

In Figure 7.2 all individual values of the healthy subjects and the Raynaud patients are given for one parameter: the mean level during recovery of both FST and LDF.

In Table 7.5 values for sensitivity are shown for each parameter for males as well as females, when specificity was 70% for every parameter by choosing the appropriate cutoff point below which RP was diagnosed. For males and females different cutoff points were found, with lower values in females for all test parameters. The sensitivity varied between 55% and 81% for the various parameters. No single parameter was superior in the discrimination between healthy subjects and Raynaud patients. This was true both for the male and the female groups. The likelihood ratio of a positive test result varied between 1.8 (mean level during cooling LDF in males: 55/30) and 2.7 (baseline LDF in females: 81/30). The likelihood ratios of the test parameters most often used in the past, i.e. the value after 12 min of recovery[10] and of the mean level during recovery[11] are almost equal.

DISCUSSION

In this study significant differences in FST and LDF were found between healthy subjects and patients with RP before, during, and after a standardized cooling procedure. No significant differences were present between the PRP and the SRP patients. Sex differences were shown in all groups, in the healthy subjects as well as in the patients with PRP or SRP, in general with higher values in males. Therefore, all results of the finger cooling test were considered separately for males and females.

Because of the rather poor sensitivity and specificity of the different test parameters, a reliable classification of an individual as a healthy subject or as a Raynaud patient on the base of this finger cooling test alone is imposssible. In the analysis a high sensitivity (high true positive rate) rather than a high specificity of the finger cooling test was preferred. Increasing the sensitivity, however, reduces the specificity of the test. A specificity of 70% was arbitrary chosen to calculate the sensitivity. Unfortunately, with this low specificity, sensitivity was still rather poor, varying between 55 and 81%. Moreover, as the prevalence of RP is lower in less selected populations than ours, the predictive value of a positive test will then be reduced further.

Table 7.5. Cutoff values with calculated sensitivities and a specificity of 70% for finger skin temperature and laser Doppler flux (in arbitrary units, AU).

Parameter	Finger skin temperature (°C)				Laser Doppler flux (AU)			
	Males Cutoff value	Sensitivity (%)	Females Cutoff value	Sensitivity (%)	Males Cutoff value	Sensitivity (%)	Females Cutoff value	Sensitivity (%)
Baseline	34.0	71	33.1	79	33.3	69	27.0	81
End of cooling	20.1	62	19.7	72	10.0	59	7.0	79
Mean level during cooling	23.6	69	23.2	74	15.5	55	10.2	69
After 12 min recovery	32.4	77	24.2	72	26.0	69	7.0	67
End of recovery	33.1	73	25.0	65	24.0	62	9.0	68
Mean level during recovery	28.9	69	23.4	71	19.6	67	8.1	68
Mean level during test	29.1	71	25.7	75	21.8	70	13.2	67

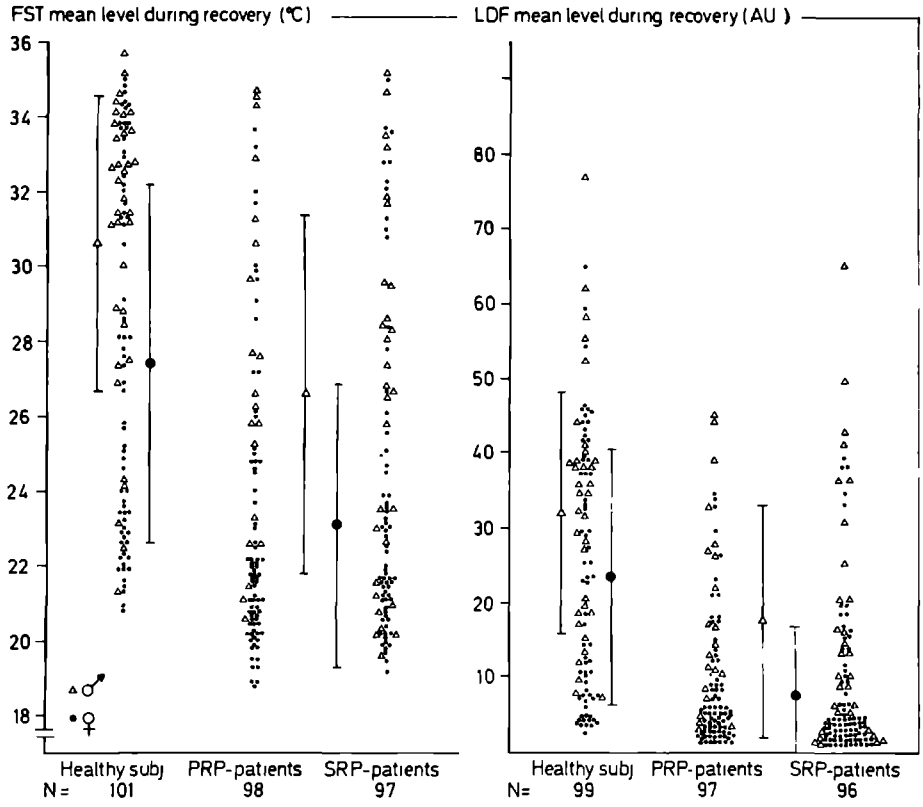

Figure 7.2. Individual values of finger skin temperature (FST) and laser Doppler flux (LDF, in arbitrary units, AU) of the mean level during the recovery period in the healthy subjects and the patients with primary Raynaud's phenomenon (PRP) and secondary Raynaud's phenomenon (SRP). Means ± SD are given for the healthy subjects and all patients with Raynaud's phenomenon, separate for males and females.

Leppert also found a considerable overlap in individual responses and a low sensitivity of finger systolic pressure measurements during local and whole body cooling in female patients with PRP and in female healthy controls.[20] Carter et al, also using finger systolic pressure measurements, showed a high accuracy of local and whole body cooling in patients with SRP but not in patients with PRP.[21] They encountered the same difficulty as we did: a considerable overlap between healthy subjects and Raynaud patients.

A critical approach towards peripheral circulatory measurements in Raynaud patients is certainly necessary.

First, cooling tests are meant to evaluate an objective response to cold provocation in Raynaud patients. However, as also experienced by other investigators,[22] we were mostly unable to elicit the typical colour changes in Raynaud patients. Besides, the reported lack of relationship between the subjective severeness of the vasospasm and the abnormal blood flow response[20] questions the reliability of provocative tests in Raynaud patients.

Second, all measurements of the peripheral circulation are hampered by the enormous changes in flow over a short period of time due to several physiologic stimuli. These factors include changes in position, perfusion pressure, heart rate or ambient temperature, vasomotion, hormonal status, previous smoking and stress. Although the strict standardization of the test reduces these stimuli, they can never be ruled out completely.

Third, some hypothesize that PRP is an extreme of the normal physiologic vasoconstriction to cold. They consider the individual's reactivity towards cold a continuum, composed of subjects without any complaints of cold digits at one end, subjects with cold digits without or with monophasic discolorations in the middle and subjects with bi- or even triphasic discolorations of the fingers at the other end. The resulting overlap in circulatory reactivity between patients with PRP and healthy subjects is then a logical consequence of this hypothesis.

Some limitations are applicable to the present study. At the time of the study we were unable to perform nailfold capillaroscopy to detect specific capillary alterations suggestive for an underlying disease. However, all patients were carefully evaluated according to a protocol with special emphasis on clinical[16] and immunological[3] signs of an underlying disease. Part of the evaluation protocol consisted of careful inspection of the nailfold capillaries by a magnifying glass that enables to detect at least some alterations in the nailfold capillaries. Follow-up was extended to at least two years before a definite classification was made.

In the period we started to perform the finger cooling tests, the 25%-calibration solution for LDF was not available yet, and therefore results are not given in

perfusion units. Recent calibration results showed an apparatus drift of only 5% over three months.

We also did not subtract the biological zero LDF. This value is relatively low (<2 AU) in healthy subjects and in PRP patients and therefore would not change LDF significantly in these groups.[23] However, in SRP patients (especially in progressive systemic sclerosis) the biological zero can be higher (up to 6 AU). This suggests that in these patients LDF after subtraction of the biological zero would become lower. This consequently would result in a better discrimination between primary and secondary Raynaud patients as described here.

Since the differences in FST and LDF between the groups might be caused by biological (age, hand volume, body mass index, amount of subcutaneous fat) or hemodynamic differences (blood pressure, heart rate, FBF, TcPO2), multiple regression was used to analyse their possible influence on the test results.

The patients with RP (especially those with SRP) were significantly older than the healthy subjects. However, age was shown not to influence the results of the finger cooling test. There were no significant differences between the healthy subjects and the patients with regard to other factors like the hand volume, the body mass index or the amount of body fat. Furthermore, none of these had any significant influence on the test results. Previously it was hypothesized that subjects with a small hand volume,[24] with a low body mass index[25] and a small amount of subcutaneous fat show an increased tendency towards cold induced vasoconstriction. These assumptions are not supported by the findings in the present study.

Blood pressure and heart rate were different between the groups. The higher blood pressure in the patient group might partly be due to the higher age. Blood pressure could theoretically influence the peripheral circulation by determining skin perfusion pressure, but neither SBP, nor DBP or MAP were found to influence FST and LDF. In males FBF, which represents mainly muscle blood flow, was higher in the healthy subjects compared with the Raynaud patients. Muscle blood flow could influence skin perfusion by a competitive mechanism. In this study, however, no such influence was found. The relatively high FBF found is explained by the use of a wrist cuff inflated at supravenous pressure which does not exclude hand blood flow.[26]

TcPO2 was different between the healthy subjects and the patients. Skin oxygen tension is the resultant of nutritional skin blood flow, and as shown the observed difference is due to the low values in patients with underlying disease, which is in accordance with their impaired capillary blood flow.[27] The lower TcPO2 found in the subgroup of SRP patients with scleroderma or CREST is in line with the increased formation of avascular fibrotic tissue with an impaired oxygen diffusion capacity of the finger skin in these diseases. Moreover, the laser light used in laser Doppler fluxmetry is increasingly dispersed by this connective tissue and therefore also

produces a lower signal.[28]

Sex differences were found not only in healthy subjects, but in patients with PRP or SRP as well. The curves in Figure 7.1 representing the finger cooling test in healthy females and in females with RP show a close resemblance, just like the curves for male healthy subjects and male patients with RP. The sex difference is most pronounced during the recovery period. Both female healthy subjects and female patients recover more slowly after cooling than male healthy subjects and male patients. Although found in healthy subjects, this sex difference was not observed in Raynaud patients in another study.[29] It could reflect either an inability to vasodilate after cold induced vasoconstriction or a prolonged vasoconstriction in females. An explanation for the observed sex difference could be the modulation of peripheral vascular reactivity by female sex hormones.[30 31] Estrogens are assumed to increase vasoconstriction, probably by up-regulation of α-adrenergic receptors.[32 33] However, in humans no direct relationship between levels of circulating female sex hormones and skin vascular reactivity has been shown yet.

In conclusion, although FST and LDF during a standardized cooling procedure were significantly lower in patients with RP in comparison with healthy subjects, the pronounced overlap limits the diagnostic value in individual cases. However, when comparing groups in pathophysiologic or in therapeutic studies this finger cooling test can be useful. Not only in healthy subjects, but also in Raynaud patients, sex should be considered when interpreting results of cold provocation tests. Consequently different cutoff points in diagnostic test parameters should be used for male and female patients.

Acknowledgements

We thank J Reyenga, research nurse, for assistance in performing the finger cooling tests. RWMM Jansen is thanked for assistance in data processing.

REFERENCES

1 Tuffanelli DL, Winkelman RK. Systemic sclerosis: a clinical study of 727 cases. Arch Dermatol 1961;84:359-71.
2 Fessel WJ. Systemic lupus erythematosis in the community. Arch Intern Med 1974;134:1017-35.
3 Wollersheim H, Thien Th, Hoet MH, van Venrooy WJ. The diagnostic value of several immunologic tests for anti-nuclear antibody in predicting the development of connective tissue disease in patients presenting with Raynaud's phenomenon. Eur J Clin Invest 1989;19:535-41.

4 Kallenberg CGM, Wouda AA, Hoet MH, van Venrooy WJ. Development of connective tissue disease in patients presenting with Raynaud's phenomenon: a six years follow up with emphasis on the predictive value of antinuclear antibodies as detected by immunoblotting. Ann Rheum Dis 1988;47:634-41.
5 Lafferty K, de Trafford JC, Roberts VC, Cotton LT. Raynaud's phenomenon and thermal entrainment: an objective test. Br Med J 1983;286:90-2.
6 Nielsen SL, Lassen NA. Measurement of digital blood pressure after local cooling. J Appl Physiol 1977;43:907-12.
7 Wouda AA. Raynaud's phenomenon. Photoelectric plethysmography of the fingers of persons with and without Raynaud's phenomenon during cooling and warming up. Acta Med Scand 1977;201:519-23.
8 Engelhart M, Kristensen JK. Raynaud's phenomenon: blood supply to fingers during indirect cooling evaluated by laser Doppler flowmetry. Clin Physiol 1986;6:481-8.
9 Mahler F, Saner H, Boss C, Annaheim M. Local cold exposure test for capillaroscopic examination of patients with Raynaud's syndrome. Microvasc Res 1987;33:422-7.
10 Cleophas T, Fennis J, van 't Laar A. Finger skin temperature after a cooling test. Influence of air temperature and cigarette smoking. J Appl Physiol 1982;52:1167-71.
11 Wollersheim H, Thien Th, Fennis J, van Elteren Ph, van 't Laar A. Double-blind, placebo-controlled study of prazosin in Raynaud's phenomenon. Clin Pharmacol Ther 1986;40:219-25.
12 van Heereveld H, Wollersheim H, Gough K, Thien Th. Intravenous nicardipine in Raynaud's phenomenon: a controlled trial. J Cardiovasc Pharmacol 1988;11:68-74.
13 Wollersheim H, Th Thien, van 't Laar A. Nifedipine in primary Raynaud's phenomenon and in scleroderma: oral versus sublingual hemodynamic effects. J Clin Pharmacol 1987;27:907-13.
14 Cooke JP, Creager MA, Osmundson PJ, Shepherd JT. Sex differences in control of cutaneous blood flow. Circulation 1990;82:1607-15.
15 Allen EV, Brown GE. Raynaud's disease, a critical review of minimal requisites for diagnosis. Am J Med Sci 1932;183:187-200.
16 Wollersheim H, Thien Th. The diagnostic value of clinical signs and symptoms in patients with Raynaud's phenomenon. A cross-sectional study. Neth J Med 1990;37:171-82.
17 Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skin fold thickness. Br J Nutr 1974;32:77-97.
18 Hunyor SN, Flynn JM, Cochineas C. Comparison of various sphygmomanometers with intra-arterial blood pressure readings. Br Med J 1978;2:159-62.
19 Sox HC. Probability theory in the use of diagnostic tests. Ann Intern Med 1986;104:60-6.
20 Leppert J. Limitation of finger systolic pressure measurement as a diagnostic test for primary Raynaud's phenomenon in a female population. Clin Physiol 1989;9:457-65.
21 Carter SA, Dean E, Kroeger EA. Apparent finger systolic pressures during cooling in patients with Raynaud's syndrome: Circulation 1988;5:988-96.
22 Coffman JD, Davies WT. Vasospastic diseases: A review. Prog Cardiovasc Dis 1975;18:123-46.
23 Caspary L, Creutzig A, Alexander K. Biological zero in laser Doppler fluxmetry. Int J Microcirc Clin Exp 1988;7:367-71.
24 Wouda AA. Raynaud's phenomenon. Acta Med Scand 1977;201:519-23.
25 Keil JE, Maricq HR, Weinrich MC, McGregor AR, Diat F. Demographic, social and clinical correlates of Raynaud phenomenon. Int J Epidemiol 1991;20:221-4.
26 Lenders J, Janssen GJ, Smits P, Thien Th. Role of the wrist cuff in forearm plethysmography. Clin Sci 1991;80:413-7.
27 Wollersheim H, Thien Th. Transcutaneous pO2 measurements in Raynaud's phenomenon: value and limitations. Int J Microcirc Clin Exp 1988;7:357-66.

28 Gush RJ, King TA, Jayson MIV. Aspects of laser light scattering from skin tissue with application to laser Doppler blood flow measurement. Phys Med Biol 1984;29:1463-76.
29 van de Wal HJCM, Wijn Pff, van Lier HJJ, Kneepkens WGHJ, Skotnicki. Noninvasive henodynamic asssessment of vasospasm in patients with primary Raynaud's phenomenon. Angiology 1987;38:315-32.
30 Altura B. Sex and oestrogen and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J Pharmacol Exp Ther 1975;193:403-12.
31 Altura BM, Altura BT. Influence of sex hormones, oral contraceptives and pregnancy on vascular smooth muscle and its reactivity. In: Carrier O, Shibata S, ed. Factors influencing vascular reactivity. Tokyo: Igaku-Shoin 1977;221-54.
32 Gisclard V, Flavahan NA, Vanhoutte PM. Alpha-adrenergic responses of blood vessels of rabbits after ovariectomy and administration of 17ß-estradiol. J Pharmacol Exp Ther 1987;240:466-70.
33 Colucci WS, Gimbrone MA, McLaughlin MK, Halpern W, Alexander RW. Increased vascular catecholamine sensitivity and α-adrenergic receptor affinity in female and estrogen-treated male rats. Circ Res 1982;50:805-11.

CHAPTER 8

CHANGES IN SKIN BLOOD FLOW DURING THE MENSTRUAL CYCLE: THE INFLUENCE OF THE MENSTRUAL CYCLE ON THE PERIPHERAL CIRCULATION IN HEALTHY FEMALE VOLUNTEERS

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Clinical Science 1990;78:527-32

ABSTRACT

It is known that females have a lower skin perfusion than males. In women there are also differences in blood flow at different reproductive stages of their lives. As an initial investigation of the possible contribution of sex hormones to these differences, we studied skin and forearm blood flow during the natural changes in hormone levels which occur during the menstrual cycle. Thirty-one healthy female volunteers were studied. The effect of a standardized finger cooling test (immersion of a gloved hand in a 16°C water bath) on finger skin temperature and on laser Doppler flux in the finger, and forearm blood flow (strain gauge venous occlusion plethysmography) was assessed at four different times during one cycle: during menstruation, 1 day before ovulation, 2 days after ovulation and at the mid-luteal phase. Test days were determined by daily measurements of basal body temperature and were confirmed afterwards by determinations of serum luteinizing hormone, follicle-stimulating hormone, 17β-oestradiol and progesterone. Peripheral skin circulation varied significantly within one menstrual cycle. The extremes were a mean finger skin temperature of 25.9 ± 3.0°C in the luteal phase compared with 28.4 ± 3.7°C in the pre-ovulatory phase (p=0.002). The respective values for the mean laser Doppler flux were 18.4 ± 10.9 compared with 29.2 ± 16.4 arbitrary units (p=0.003). Baseline forearm muscle blood flow also varied significantly (p=0.04) within one menstrual cycle, with low values in the menstrual phase compared with the other phases. We have shown that peripheral skin circulation and forearm muscle blood flow exhibit significant variability during the hormonal changes in a menstrual cycle.

INTRODUCTION

There are several observations indicating that sex hormones affect peripheral blood flow. Healthy women, in their fertile phase of life, show up to 50% lower peripheral skin blood flow than men, whereas there is no difference between the sexes before the menarche and after the menopause.[1,2] The severity and frequency of cold-induced vasospastic attacks and the measured skin blood flow vary within the different phases of the menstrual cycle.[3,4,5,6] Disturbances in the peripheral circulation are not equally distributed between the sexes either. Vasospastic diseases, such as Raynaud's phenomenon, migraine and variant angina, are more common in women, whereas men more often suffer from atherosclerotic vascular diseases. In primary Raynaud's phenomenon the female/male ratio ranges between 2 and 9.[3,7,8,9] The prevalence varies from 5% up to 20% in otherwise healthy females.[7,10,11,12] The onset of vasospastic complaints shortly after the menarche,[3,8] the improvement of symptoms after the menopause[7,10] and the decrease in frequency and severity of the attacks during pregnancy, all suggest the influence of female sex hormones. Moreover, it is

suggested that oral contraceptives worsen the complaints.[13 14]

Unfortunately there are no studies showing a correlation between naturally occurring levels of sex hormones and changes in blood flow. We investigated the possible correlation between the level of sex hormones and peripheral blood flow by measuring vascular reactivity during the normal changes in hormone levels which occur during the menstrual cycle of healthy women.

SUBJECTS AND METHODS

Subjects

We selected 31 healthy female volunteers, 15-45 yr of age, with a regular menstrual cycle of between 26 and 32 days during the previous 4 months. None used medications or oral contraceptives, and no symptoms of Raynaud's phenomenon were present. They all gave informed consent to the protocol, which was approved by the hospital ethical committee.

Methods

On four occasions within one menstrual cycle, a provocative finger cooling test (FCT) was performed in order to quantify cold-induced vasoconstriction. Previously, we have demonstrated that finger skin temperature (FST) recovery in this test has a good reproducibility if room temperature and other factors influencing the finger perfusion are standardized.[15] For this reason, all volunteers were asked to abstain from smoking for 24 hr, from caffeine- and alcohol-containing beverages for 12 hr and to fast for at least 2 hr before the test. All FCTs were performed at the same time of the day, in a climate room (ambient temperature: 24.7 $\pm$ 0.3°C; humidity: 59 $\pm$ 2%) with the subjects in a comfortable supine position, their arms at heart level. After an acclimatization period of at least 20 min, the following measurements were performed. Systolic (SBP) and diastolic (DBP) blood pressure (in mmHg) were measured in the left arm by Arteriosonde (Roche Medical Electronics Inc, Oranjeburg, NJ, USA)[16] and heart rate (HR, in beats/minute) was calculated from an electrocardiogram strip. Forearm muscle blood flow (FBF, in ml/100 ml/min) was measured at the left forearm by strain gauge venous occlusion plethysmography (Loosco BVP 96, Hoekloos, Amsterdam, The Netherlands), using a wrist-cuff (inflated to 40-50 mmHg) to exclude the venous return from the hand. FST (in °C) was measured on the second volar fingertip of the right hand (Thermocouple, Ellab Instruments, Copenhagen, Denmark). Laser Doppler flux (LDF, in arbitrary units) was measured on the third volar fingertip of the right hand (Periflux Pf-1d, Perimed, Stockholm, Sweden) after zero calibration. For all measurements the average of three

pre-test values obtained after the acclimatization period was used as the baseline value. After this, the gloved right hand was immersed in a water bath at 16°C for 5 min, during which FST and LDF were recorded every min. During a recovery period of 20 min FST and LDF were measured every 2 min, whereas FBF, HR, SBP and DBP were measured separately every 10 min.

The first FCT took place on the second or third day of the cycle, during menstruation (menstrual phase). The second FCT was performed 1 day before the expected day of ovulation (pre-ovulatory phase). The third test was carried out 2 days after ovulation (post-ovulatory phase) and the last FCT took place 8 days after ovulation, in the mid-luteal phase. The menstrual phase exhibits low values of all the sex hormones measured. In the pre-ovulatory phase 17ß-oestradiol levels are at their peak, progesterone levels are slightly rising and levels of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) are increasing. After ovulation, in the post-ovulatory phase, 17ß-oestradiol levels are falling, progesterone levels are still rising and levels of both LH and FSH are low. In the mid-luteal phase 17ß-oestradiol levels are again at their peak, progesterone levels are at their peak and levels of both LH and FSH are still low (see Table 8.1). The duration of previous menstrual cycles was used to determine the days on which an FCT should be performed. It was assumed that the luteal phase had a duration of 14 days and that ovulation took place 14 days before the first day of the next menstruation.

Table 8.1. Hormone levels during the four phases of the menstrual cycle. Results are shown as mean (range).

Phase	Menstrual n=31	Pre-ovulatory n=30	Post-ovulatory n=23	Mid-luteal n=26
17ß-Oestradiol (pmol/l)	207 (75-330)	1081 (190-2000)	550 (260-900)	733 (380-1000)
Progesterone (nmol/l)	2.4 (1.3-5.4)	4.6 (1.3-13.0)	24.6 (4.7-64.0)	68.0 (23.0-120.0)
LH (IU/l)	9.4 (5.2-16.0)	26.8 (8.2-72.0)	15.5 (4.0-38.0)	10.1 4.5-20.0)
FSH (IU/l)	5.1 (1.1-9.2)	3.1 (2.7-16.0)	4.9 (1.5-9.2)	2.9 (1.1-6.1)

All women recorded their basal body temperature during the investigation cycle starting on the first day of the menstrual period. Rectal temperature (during at least 3 min) was measured each morning at the same time, before leaving the bed. The last low point in the temperature curve before the rise towards a hyperthermic plateau started, was considered the day of ovulation. In case of a difference between the

expected day of ovulation and the recorded basal body temperature extra FCTs were carried out.

On the days on which an FCT was performed, blood was sampled by venepuncture of the antecubital vein for determination of the serum levels of FSH, LH, 17ß-oestradiol and progesterone. The samples were frozen (-20°C) and the hormone levels were determined later by radioimmunoassay.[17] [18] After the investigation cycle, the available hormonal data and basal body temperature charts were submitted to an independent, experienced observer, who verified the phase of the cycle.

To control for a number of other influences on skin blood flow that might explain the differences in peripheral blood flow between the sexes, we also determined the body weight (in kg), the height (in m) and calculated the amount of subcutaneous fat (in %) by measuring the skinfold thickness at four standardized places of the body.[19] The hand volume was determined (in ml) by immersing the right hand up to the wrist in a fixed amount of water.

The mean arterial pressure (MAP, in mmHg) was calculated from the formula MAP=(SBP+2DBP)/3, and the forearm vascular resistance (FVR, in arbitrary units) was obtained by dividing MAP by FBF. The Quetelet index was calculated by dividing weight by (height)^2 and was expressed in kg/m^2.

Data Analysis and Statistics

The data from those tests which were retrospectively confirmed to be correctly timed were used. In 19 of the original 31 subjects, we obtained results in all four desired phases of the cycle. In the remaining 12 subjects, tests, mostly in the post-ovulatory phase, appeared not to have been performed at the appropriate time. Results are given for, and statistical analysis was performed on, all the data from correctly timed tests (menstrual phase, n=31; pre-ovulatory phase, n=30; post-ovulatory phase, n=23; mid-luteal phase, n=26).

To obtain a measure of the overall level of FST and LDF during the FCT, the area under the curve during the test was calculated and divided by the total length (min) of the test period. The resulting quotient is a weighted average of the periods for which each value is representative. It is called 'mean (immersion and recovery)'. We used the same procedure for the cooling period (5 min), the 'mean (immersion only)' and for the level of FST and LDF during the recovery period (20 min), the 'mean (recovery only)'.[20] The absolute changes in FST and LDF from baseline values were calculated as well.

We also made an approximation of the course of FST over time (during recovery) by using an exponential model.[21] For each test the change in FST after recovery with respect to the baseline value was approximated by a simple exponentional model:

baseline-FST(t 'after recovery')=a $\exp^{(-bt)}$, where t is the time in min from the start of recovery, a is the temperature drop during immersion, and b is the rate of recovery. The estimated values for a and b were used as parameters for the relation between FST and the four phases. Statistical comparisons between the test results in the different phases were performed by an analysis of variance, mixed model, with subject as the random factor and phase as the fixed factor, and allowing some empty cells. Pairwise comparison between the phases was performed by using the method of Scheffé.

For each subject we calculated the Kendall correlation coefficient between the hormone levels and mean (recovery only) FST, and to investigate the consistency of these relations in all women together, we used a signed rank test. Correlations between hormone levels and FST values for all women were calculated by Pearson correlation coefficients. A p value of less than 0.05 (two-tailed) was considered to be significant. All results are given as means $\pm$ SD, unless indicated otherwise.

RESULTS

We investigated 31 females with an age of 25.0 $\pm$ 4.1 yr, a cycle length of 29.3 $\pm$ 3.5 days, a body weight of 62.0 $\pm$ 9.6 kg, a height of 1.67 $\pm$ 0.07 m, a Quetelet index of 21.5 $\pm$ 2.5 kg/m^2, an amount of subcutaneous fat of 26 $\pm$ 4% of total body mass, and a hand volume of 312 $\pm$ 51 ml.

Table 8.1 gives the means and the range of the levels of the sex hormones in the different phases of the menstrual cycle. In Figures 8.1 and 8.2, the mean of all measurements of FST and LDF during the whole FCT is shown in all four phases of the cycle. Table 8.2 shows the FST in the different phases. There was no significant difference in the baseline FST between the four phases. However, on further analysis significant variations were found during the menstrual cycle (all $p<0.005$) for the mean (immersion and recovery) FST, the mean (immersion only) FST and the mean (recovery only) FST. Significant Scheffé contrasts were observed between the mid-luteal phase and the pre-ovulatory phase. Also, in the exponentional model, significant differences for the temperature drop a ($p=0.03$) and the recovery rate b ($p=0.005$) were calculated. Again, the significant contrast was between the mid-luteal phase and the pre-ovulatory phase for b, and between the menstrual phase and the post-ovulatory phase for a. Table 8.2 also shows that the LDF values varied significantly during one menstrual cycle. The baseline LDF showed a significant difference ($p=0.04$), as did the mean (immersion and recovery) LDF, the mean (immersion only) LDF and the mean (recovery only) LDF (all $p<0.005$). Again the significant contrasts for all the parameters were found between the mid-luteal phase and the pre-ovulatory phase. Since the baseline values were already different, the changes were also expressed as absolute changes from baseline and then all the mean LDF values during the different

phases were no longer significant. Table 8.2 gives only the most representative, the mean (recovery only) LDF value.

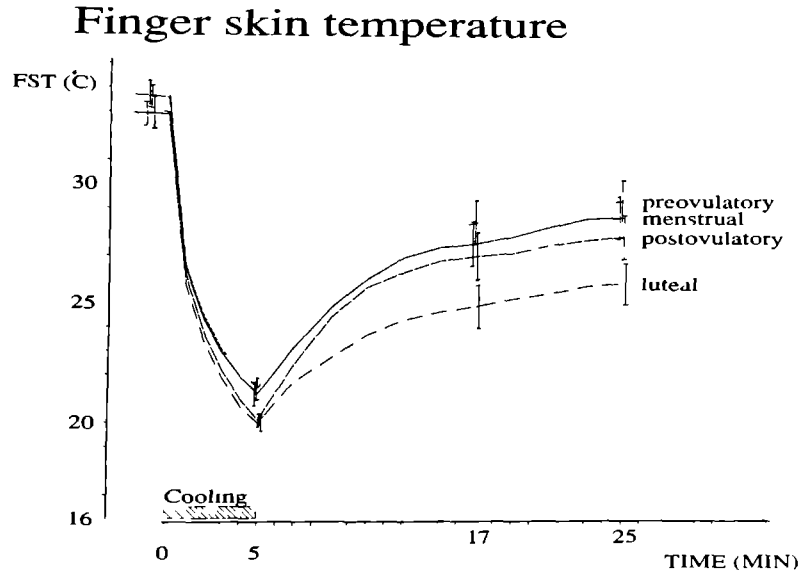

Figure 8.1. FST before, during and after cooling, in the four different phases of the menstrual cycle. Results are means ± SEM.

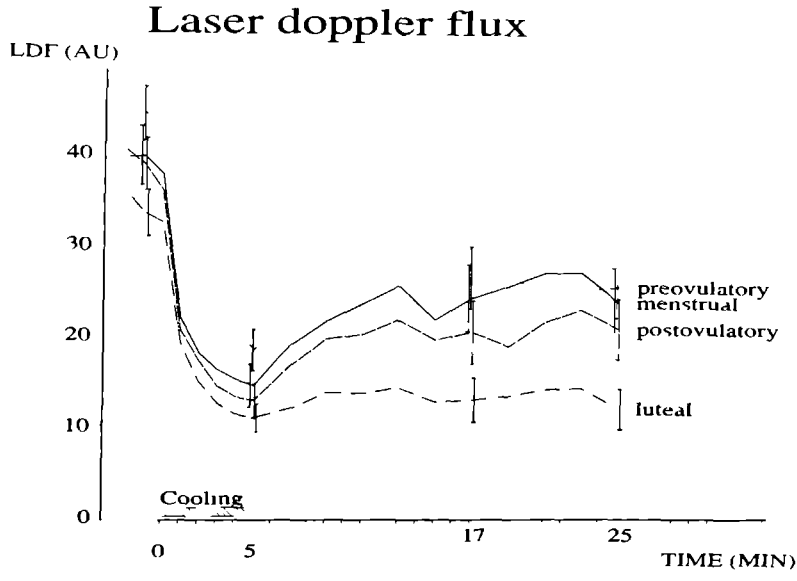

Figure 8.2. LDF, in arbitrary units (AU) before, during and after cooling, in the four different phases of the menstrual cycle. Results are means ± SEM.

Table 8.2. FST and LDF during the four phases of the menstrual cycle. Results are means ± SD for n tests. Abbreviation: NS, not significant. P values refer to the analysis of variance for differences between phases. Comparisons refer to significant ($p < 0.05$) Scheffé comparisons.

	Menstrual (M, n=31)	Pre-ovulatory (PrO, n=30)	Post-ovulatory (PO, n=23)	Mid-luteal (M, n=26)	P value	Comparisons
FST (°C)						
Baseline value	32.9 ± 2.8	33.8 ± 2.4	33.6 ± 2.0	33.1 ± 2.6	NS	
Mean (immersion and recovery)	27.7 ± 3.6	28.4 ± 3.7	27.2 ± 3.2	25.9 ± 3.0	0.002	PrO vs L
Mean (immersion only)	24.5 ± 2.1	24.8 ± 1.8	24.0 ± 1.4	23.6 ± 1.5	0.004	PrO vs L
Mean (recovery only)	26.7 ± 4.6	27.4 ± 4.9	25.9 ± 4.3	24.1 ± 3.9	<0.001	PrO and M vs L
Baseline minus mean (recovery only)	6.3 ± 3.5	6.5 ± 4.0	7.8 ± 3.5	9.0 ± 3.6	<0.001	M vs L
Temperature drop a (°C)	11.7 ± 3.0	12.3 ± 2.6	13.5 ± 1.9	12.9 ± 2.3	0.03	M vs PO
Recovery rate b (min^{-1})	0.12 ± 0.13	0.18 ± 0.21	0.10 ± 0.14	0.07 ±0.12	0.005	PrO vs L
LDF (arbitrary units)						
Baseline value	39.0 ± 17.9	43.9 ± 15.8	38.3 ± 13.4	33.9 ±12.8	0.04	PrO vs L
Mean (immersion and recovery)	26.1 ± 16.7	29.2 ± 16.4	22.5 ± 12.3	18.4 ±10.9	0.003	PrO vs L
Mean (immersion only)	19.5 ± 12.5	25.7 ± 14.4	17.8 ± 9.9	16.1 ± 9.0	0.001	PrO and PO vs L
Mean (recovery only)	23.4 ± 19.1	25.0 ± 17.7	18.6 ± 14.5	13.7 ±12.5	0.002	PrO and M vs L
Baseline minus mean (recovery only)	15.6 ± 14.2	18.9 ± 12.2	19.1 ± 13.6	20.3 ±13.4	NS	

Table 8.3. Baseline values of blood pressure, heart rate, FBF and FVR in the four phases of the menstrual cycle. Results are means ± SD for n tests. Abbreviation: NS, not significant. P values refer to the analysis of variance for differences between phases.

Phase	Menstrual n=31	Pre-ovulatory n=30	Post-ovulatory n=23	Mid-luteal n=26	P value
Blood pressure (mmHg)					
Systolic	103.7 ± 6.5	103.8 ± 6.7	102.7 ± 5.4	103.1 ± 6.1	NS
Diastolic	69.8 ± 6.5	69.4 ± 6.0	68.1 ± 5.7	68.3 ± 5.6	NS
Mean	81.1 ± 6.0	80.9 ± 5.7	79.6 ± 5.1	79.9 ± 5.3	NS
Heart rate (beats/min)	63.5 ± 8.6	64.8 ± 9.5	63.8 ± 10.0	64.7 ± 9.0	NS
FBF (ml/100ml/min)	2.90 ± 1.5	3.79 ± 2.0	3.93 ± 1.7	3.88 ± 1.8	0.041
FVR (arbitrary units)	37.6 ± 27.3	26.3 ± 10.9	24.1 ± 9.4	24.8 ± 10.2	0.002

Table 8.3 gives further baseline haemodynamic data. The baseline measurements at the four phases of the menstrual cycle were similar for blood pressure and HR, but different for FBF ($p<0.05$) and calculated FVR ($p=0.002$). The most striking finding here was the lower FBF in the menstrual phase compared with more or less similar values in the other three phases. As expected, the baseline FVR was increased in the menstrual phase in comparison with the three other phases. During the FCT no changes in blood pressure, HR and forearm parameters were detected in any of the four phases (data not shown).

We looked for correlations between the results of the FCT (expressed as mean (recovery only) FST) and the levels of 17ß-oestradiol, progesterone, LH, FSH and the 17ß-oestradiol/progesterone ratio. We did not see any correlation between these hormones (for all subjects and all phases) and the test results. We further calculated correlations between the sex hormones and the test results in the different phases. We found a negative correlation ($r=-0.48$, $n=26$, $p=0.012$) between the level of 17ß-oestradiol and the mean (recovery only) FST in the mid-luteal phase but not in the other three phases.

Within subjects we found a positive correlation between the 17ß-oestradiol/progesterone ratio and the mean (recovery only) FST, by calculating the Kendall correlation coefficients between hormone level and FST for each woman. The mean of these correlation coefficients was 0.38, with $p<0.05$ in the signed rank test. No significant correlations were found between the results of the FCT and the Quetelet index, the amount of subcutaneous fat or hand volume.

DISCUSSION

This study shows an effect of menstrual cycle phase on the peripheral skin perfusion and FBF. In the luteal phase, finger skin perfusion shows the greatest cold-induced vasoconstriction and the slowest recovery afterwards as compared with the other phases of the cycle. The adaptation to changes in environmental temperatures and emotions results in large physiological fluctuations in peripheral skin blood flow, which causes difficulties in measuring this parameter. Consequently, both methods, FST and LDF, have a high SD.

Lafferty et al.[4] and Terregino & Seibold[5] also found cyclic differences in finger skin blood flow, but in a different pattern from that which we found. In contrast with these studies, we studied a much larger group of subjects, our FCT was strictly standardised[15] and we used the combined determination of serum 17ß-oestradiol, progesterone, LH and FSH and a basal body temperature chart to determine the correct timing of ovulation.

We have also shown a cyclic change in FBF, consisting mainly of muscle flow. This appeared to be low in the menstrual phase, consistent with other reports of a

lower blood flow in the calf during menstruation.[22]

The particular role played by sex hormones in the regulation of the peripheral circulation is not yet known. There might be a direct influence on the blood vessel wall or through other peripheral regulation mechanisms, or an indirect systemic hormonal action causing a cyclic pattern in females. Besides, non-hormonal factors might be responsible for differences in women compared with men. The influence of natural and synthetic oestrogens on different tissues and arteries and veins has been the object of studies both in vitro or in vivo, mostly in animals. Observations are largely dependent on the species and the tissue used and cannot be automatically extrapolated to other species or tissues. Natural and synthetic oestrogens may sometimes exhibit a completely opposite effect on the same target.[23 24 25 26]

The regulation of cutaneous blood flow and the response to cold challenge is mainly under sympathetic nervous system control. The vasomotor tone within skin vessels, particularly the arteriovenous anastomoses, is reflexly influenced by both local circulating mediators and the sympathetic nervous system. Nutritional capillary skin flow is more constant and is influenced by local factors rather than directly by sympathetic nerves. The sympathetic nervous system releases catecholamines into the synaptic cleft that may stimulate vasoconstrictive α_2-adrenoceptors and vasodilating β_2-adrenoceptors. Some investigators have found cyclical changes in platelet α_2-adrenoceptors,[27] others found cyclical changes only in β_2-adrenoreceptors,[28] and some no change at all.[29] Studies point to an influence of oestrogens on the sympathetic nervous system and there is evidence that oestrogens induce an up-regulation of (vasoconstrictive) α_2-adrenoceptors.[30 31 32] Experiments in arteries and veins of rats revealed a dose-dependent reactivity of oestrogens, with vasoconstriction after low and vasodilation after high dosage.[33 34] Although much less investigated, the same seems true for progesterone, leading to a vasoconstrictive as well as a vasodilatory effect, the latter possibly by influencing the vasodilating ß-adrenoceptors.[26 34] The most satisfying hypothesis is that the cyclical 17ß-oestradiol/progesterone ratio and its continuous fluctuation is of major importance in the vascular responsiveness in women. Indeed, we did find a correlation between this ratio and the blood flow, although it was a weak.

The sex hormonal influenced premenstrual fluid and electrolyte retention or the progesterone-induced post-ovulatory elevated core temperature may play an indirect role in the regulation of the peripheral circulation. Eventually, non-hormonal factors may have an influence on the skin circulation. For example, a low Quetelet index or a low amount of subcutaneous fat may lead to less isolation against low temperature. Also, a low hand volume may imply that a relatively greater surface is in contact with the environmental temperature.[35] Yet we could not establish that any of these factors influenced the FCT.

In conclusion, this study has demonstrated that during the menstrual cycle with its

natural changes in sex hormone levels, the peripheral circulation and its response to cold changes. These differences are worth considering when the peripheral circulation of females is investigated without knowledge of the phase of the menstrual cycle.

Acknowledgement

We thank the staff of the Laboratory of Gynaecology (Head: Dr CMG Thomas) for careful measurement of the sex hormones.

REFERENCES

1 Van de Wal HJCM, Wijn PFF, van Lier HJJ, Kneepkens WGHJ, Skotnicki SH. Noninvasive hemodynamic assessment of vasospasm in patients with primary Raynaud's phenomenon. Angiology 1987;38:315-32.
2 Bollinger A, Schlumpf M. Finger blood flow in healthy subjects of different age and sex in patients with primary Raynaud's disease. Acta Chir Scand 1975;suppl:42-7.
3 Trafford de JC, Lafferty K, Potter CE, Roberts VC, Cotton LT. An epidemiological survey of Raynaud's phenomenon. Eur J Vasc Surg 1988;2:167-70.
4 Lafferty K, de Trafford JC, Potter VC, Cotton LT. Reflex vascular responses in the finger to contralateral thermal stimuli during the normal menstrual cycle: a hormonal basis to Raynaud's phenomenon? Clin Sci 1985;68:639-45.
5 Terregino CA, Seibold FACA. Influence of the menstrual cycle on Raynaud's phenomenon and on cold tolerance in normal women. Angiology 1985;36:88-95.
6 Tooke JE, Tindall H, McNicol GP. The influence of a combined oral contraceptive pill and menstrual cycle phase on digital microvascular haemodynamics. Clin Sci 1981;61:91-5.
7 Heslop J, Coggen D, Acheson ED. The prevalence of intermittent digital ischaemia (RP) in a general practice. J Roy Coll Gen Pract 1983;33:85-9.
8 Spittell JA. Raynaud's phenomenon and allied vasospastic disorders. In: Jurgens JL, Spittell JA, Fairbairn JF, eds. Peripheral Vascular Diseases. Philadelphia, London, Toronto: Saunders Company, 1980:588.
9 Gifford RW, Hines EA. Raynaud's disease among women and girls. Circulation 1957;16:1012-21.
10 Hines AE, Christensen NA. Raynaud's disease among men. JAMA 1945;129:1-4.
11 Olsen N, Nielsen L. Prevalence of primary Raynaud phenomena in young females. Scand J Clin Lab Invest 1978;37:761-4.
12 Maricq HR, Weinrich MC, Keil EK, Leroy EW. Prevalence of Raynaud phenomenon in the general population. J Chron Dis 1986;39:423-7.
13 Eastcott HHG. Raynaud's disease and the contraceptive pill. Br Med J 1976;2:477.
14 Jarrett PEM. Raynaud's disease and oral contraceptives. Br Med J 1976;2:699.
15 Cleophas TJM, Fennis JFM, van 't Laar A. Fingertemperature after a FCT. Influence of airtemperature and cigarette smoking. J Appl Physiol 1982;52:1167-71.
16 Hunyor SN, Flynn JM, Cochineas C. Comparison of various sphygmomanometers with intra-arterial blood-pressure readings. Br Med J 1978;2:159-62.
17 Thomas CMG, Corbey RS, Rolland R. Assessment of unconjugated oestradiol and progesteron serum levels throughout pregnancy in normal women and in women with hyperprolactinaemia who conceived after bromocriptine treatment. Acta Endocrinol 1977;86:405-14.

18 Van Geelen JM, Doesburg WH, Thomas CMG, Martin CB. Urodynamic studies in the normal menstrual cycle; relationship between hormonal changes during the menstrual cycle and the urethral pressure. Am J Obstet Gynecol 1981;141:384-92.
19 Durnin JVGA, Womersley J. Bodyfat assessed from total body density and its estimation from skinfold thickness. Br J Nutr 1974;32:77-97.
20 Wollersheim H, Thien Th, Fennis J, van Elteren Ph, van 't Laar A. Double-blind, placebo-controlled study of prazosin in Raynaud's phenomenon. Clin Pharmacol Ther 1986;40:219-25.
21 Lenders JWM, Salemans J, de Boo Th, Lemmens WAJ, Thien Th, van 't Laar A. The influence of intrinsic sympathomimetic activity and beta-1-receptor selectivity on the recovery of finger skin temperature after finger cooling in normotensive subjects. Clin Pharmacol Ther 1986;39:353-7.
22 Keates JS, Fitzgerald DE. Limb volume and blood flow changes during the menstrual cycle. Angiology 1969;20:624-7.
23 Mishra N, Hamilton CA, Jones CR, Leslie C, Reid JL. α-Adrenoceptor changes after oestrogen treatment in platelets and other tissues in female rabbits. Clin Sci 1985;69:235-8.
24 Gisclard V, Flavahan NA, Vanhoutte PM. α-Adrenergic responses of blood vessels of rabbits after ovariectomy and administration of 17ß-estradiol. J Pharmacol Exp Ther 1987;240:466-70.
25 Roberts JM, Insel PA, Goldfien A. Regulation of myometrial adrenoceptors and adrenergic responses by sex steroids. Mol Pharmacol 1980;20:52-8.
26 Altura BM, Altura BT. Influence of sexhormones, oral contraceptives and pregnancy on vascular muscle and its reactivity. In: Carrier O, Shibata S, eds. Factors influencing vascular reactivity. Tokyo: Igaku-Shoin, 1977:221-54.
27 Jones SB, Bylund DB, Rieser CA, Shekim WO, Beyer JA, Carr GW. α_2-Adrenergic receptor binding in human platelets: alterations during the menstrual cycle. Clin Pharmacol Ther 1983;34:90-6.
28 Stowell LI, McIntosh CJ, Cooke R, Ellis PM. Adrenoceptor and imipramine receptor binding during the menstrual cycle. Acta Psychiatr Scand 1988;78:366-8.
29 Sundaresan PR, Madan MK, Kelvie SL, Weintraub M. Platelet α_2-adrenoceptors and the menstrual cycle. Clin Pharmacol Ther 1985;37:337-42.
30 Altura B. Sex and estrogen and responsiveness of terminal arteriole to neurohypophyseal hormones and catecholamines. J Pharmacol Exp Ther 1975;193:403-12.
31 Colucci WS, Gimbrone MA, McLaughlin MK, Halpern W, Alexander RW. Increased vascular catecholamine sensitivity and α-adrenergic receptor affinity in female and estrogen-treated male rats. Circ Res 1982;50:805-11.
32 Roberts JM, Insel PA, Goldfien RD, Goldfien A. α-Adrenoceptors but not ß-adrenoceptors increase in rabbit uterus with estrogen. Nature 1977;270:624-5.
33 Kondo K, Okuno T, Eguchi T, Yashui T, Suzuki H, Nagahama S, Saruta T. Vascular action of high dose estrogen in rats. Endocrinol Jpn 1980;27:307-13.
34 McCalden TA. The inhibitory action of oestradiol-17ß and progesterone on venous smooth muscle. Br J Pharmacol 1975;53:183-92.
35 Wouda AA. Raynaud's Phenomenon. Acta Med Scand 1977;201:519-23.

CHAPTER 9

SKIN VASCULAR REACTIVITY IN HEALTHY SUBJECTS: INFLUENCE OF HORMONAL STATUS

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Journal of Applied Physiology, in press

ABSTRACT

To investigate the influence of the hormonal status on skin vascular reactivity 18 males, 18 women using oral contraceptives (oc), 17 premenopausal and 18 postmenopausal women were studied. Finger skin temperature (FST in °C) and laser Doppler flux (LDF in perfusion units) were measured during heating (45°C water bath) and cooling (15°C) followed by a subsequent recovery period. Maximal heat-induced vasodilation was significantly higher in women using oc and in premenopausal women when compared with males. During cooling, FST and LDF were significantly higher in males compared with, respectively, women using oc and premenopausal women. FST was also higher in postmenopausal women compared with women using oc. During recovery, FST was significantly higher in males than in women using oc. LDF was significantly higher in males than in women using oc and in premenopausal women. These findings are consistent with a less pronounced and less prolonged cold induced vasoconstriction in males. Other hemodynamic (such as blood pressure or heart rate) or biological factors (such as age, amount of subcutaneous fat, hand volume or body mass index) that possibly influence peripheral blood flow were found not to influence the results. In conclusion, the observed differences in vascular reactivity towards temperature changes between subjects with a different hormonal status suggest that sex hormones influence finger skin perfusion.

INTRODUCTION

Vasospastic syndromes such as Raynaud's phenomenon (RP) and migraine are more common in females than in males.[1,2] In women the severity of complaints of RP is most pronounced in the period between menarche and menopause.[1,2,3,4] Occasionally case reports suggest a negative influence of oral contraceptives (oc) on the complaints.[5]

In healthy subjects sex differences in peripheral circulation have been found as well.[6,7,8,9,10] The observed lower resting skin blood flow in females in comparison with males disappears in postmenopausal women.[6] Administration of oc induces alterations in both peripheral muscle and skin blood flow in premenopausal women.[9,11,12,13] Several investigators observed a variation in finger skin blood flow in premenopausal women in different phases of the menstrual cycle.[13,14,15,16] Although a clear and direct relationship between circulating endogenous or exogenous estrogens or progestagens has not been shown in these studies in humans, the observations suggest an influence of these female sex hormones on the peripheral circulation. In animal studies, however, synthetic as well as endogenous estrogens, progestagens and androgens have been observed to influence vascular reactivity.[17,18] In general, it is assumed that estrogens are able to enhance vascular tone, whereas progesterone either

has no effect or tends to do the reverse. The effects of testosterone, luteinizing hormone (LH) or follicle stimulating hormone (FSH) are not well documented.[18]

To investigate the influence of hormonal status on skin blood flow, four groups of healthy volunteers with a different hormonal status were studied, respectively a group of males and three groups of females: premenopausal and postmenopausal women and women using oc. As shown in Table 9.1, testosterone is present in males but both female sex hormones 17ß-estradiol and progesterone are almost absent. All women have low levels of testosterone. The menstrual phase of women is characterized by low 17ß-estradiol and progesterone after previously high levels. Use of the combined oral contraceptive pill produces high levels of synthetic estrogens and progestagens with consequent depression of FSH and LH and the endogenous production of sex hormones. After the menopause involution of the ovaries results in very low 17ß-estradiol and progesterone levels with high FSH and LH levels. We hypothesize that subjects with elevated levels of synthetic sex hormones or endogenous estradiol and progesterone show enhanced vascular reactivity compared with postmenopausal women and males. The absence of female sex hormones in males and postmenopausal women might induce less vascular reactivity compared with the other groups of females.

Table 9.1. Level of sex hormones in the four groups: women using oral contraceptives, premenopausal women, postmenopausal women and males.

	Oral contraceptives	Premeno-pausal	Post-menopausal	Males
Testosterone	low	low	low	high
17ß-Estradiol	low	low, but cyclic variation	low	low
Synthetic estrogens	high	-	-	-
Progesterone	low	low, but cyclic variation	low	low
Synthetic progestagens	high	-	-	-
FSH, LH	low	low	high	low

To quantify finger skin reactivity and to study whether the presence of estrogens and

progestagens enhances vascular reactivity in this area, a standardized finger heating and cooling test was used. In this test finger skin temperature (FST) and laser Doppler flux (LDF) are measured before and during direct local heating and cooling.

SUBJECTS AND METHODS

Subjects

We selected 71 healthy volunteers from the general population not using any vasoactive medication. None had complaints of discolorations of fingers or toes.

Four groups were distinguished: 18 males, 17 premenopausal women with regular menstrual cycles (between 26 and 32 days), 18 postmenopausal women (last menstruation at least 6 months prior to the study) and 18 premenopausal women using oc for at least 6 months (30 μg ethinylestradiol and 150 μg desogestrel in 8 females and 30 μg ethinylestradiol and 150 μg levonorgestrel in 10 females). All subjects were $\geq$ 18 yr of age. The protocol was approved by the hospital ethical committee.

Methods

In males and postmenopausal women an arbitrary day was chosen to perform a standardized finger heating and cooling test. Premenopausal women were tested in the same phase of the menstrual cycle (the 3rd, 4th, or 5th day of menstruation), because skin blood flow varies within a menstrual cycle.[14] Furthermore, the hormone levels are the least variable between individuals in this phase of the menstrual cycle. In women using oc the tests were performed during usage of the 10th to 21th pill of the strip, because it takes a minimum of 4 days to obtain maximal plasma levels of the exogenous hormones after the monthly withdrawal period. The levels of endogenous estradiol and progesterone are constantly low during the pill cycle.[19]

Factors influencing peripheral circulation were eliminated by asking all subjects to refrain from smoking for 24 hr,[20] alcohol- and caffeine-containing beverages for 12 hr and not to eat for at least 2 hr prior to the test. All tests were performed in a quiet climatized room with a constant ambient temperature of 24.5 $\pm$ 1°C and a humidity of 59 $\pm$ 0.9%. All subjects were lightly dressed and rested supine with their arms supported on heart level. All tests were performed in the morning. Mean outside temperature during the tests was 6.5 $\pm$ 3.2°C.

The amount of subcutaneous fat (% of total body mass) was determined by measuring skinfold thickness at four standardized sites of the body (in mm), after which the corresponding percentage of subcutaneous fat according to the age and sex was read from a table.[21] The hand volume (in ml) was determined by the increase in volume after immersion of the hand up to the wrist in a definite amount of water.

Length (in m) and weight (in kg) were determined.

After an equilibration period of at least 20 min systolic (SBP) and diastolic (DBP) blood pressure were measured in the left arm by an Arteriosonde (in mmHg; Roche Medical Electronics Inc., Oranjeburg, NJ, USA). The heart rate (in beats per minute) was calculated from 10 RR-intervals of an electrocardiogram-strip.

Finger skin perfusion was quantified by laser Doppler fluxmetry (LDF) and finger skin temperature (FST). LDF was measured in the center of the third volar fingertip of the right hand (in perfusion units (PU); Periflux Pf-ld, Perimed, Stockholm, Sweden). The gain was adjusted to 3, the cutoff frequency to 12 kHz and the time constant to 3 sec. LDF was continuously registered on a chart recorder and a personal computer. Flux zero calibration was performed by readjusting the pen of the recorder to zero, when the probe was fixed to a white, non-moving surface. Once a week a calibration with 25%-calibration fluid (Periflux motility standard, Perimed) was performed. LDF was calculated afterwards as the mean of a 1-min period. The biological zero was determined by arresting the circulation of the third finger during 5 min by a suprasystolic inflated cuff. The resulting LDF was readjusted to zero.

FST (in °C) was measured at the volar side of the intermediate phalanx of the third finger (Thermocouple, Ellab Instruments, Copenhagen, Denmark). The part of the thermocouple in contact with the water was isolated. FST therefore mainly represents the temperature of the finger skin and not of the surrounding water.

In order to obtain comparable baseline values, the gloved right hand was immersed in a 30°C water bath during 10 min. Preheating and precooling LDF was obtained by the average of the last 5 min. The preheating and precooling FST was the value just before respectively heating and cooling.

Heating was performed by immersion of the gloved right hand in water of 45°C during 10 min. Cooling was performed by immersing the gloved hand in water of 15°C during 5 min. During these procedures every min the average LDF and the momentary FST were registered. After cooling a subsequent recovery period of 20 min followed with the hand exposed to room temperature. During recovery LDF (average of the previous min) and FST (momentary) were registered every minute in the first 6 min and every 2 min afterwards.

Data analysis and statistics

Unless stated otherwise, results are shown as means ± SD. The mean arterial pressure (MAP; in mmHg) was calculated from the formula MAP=(SBP+2DBP)/3. The body mass index was calculated by dividing weight by height^2 and expressed as kg/m^2. The following parameters for both FST and LDF were used in the analysis: the preheating value, the maximum value during heating, the precooling value and respectively the minimum value and the percentual decrease during cooling.

Table 9.2. General characteristics of the four groups: women using oral contraceptives, premenopausal women, postmenopausal women and males. Results are means ± SD.

	Oral contraceptives (n=18)	Premeno-pausal (n=17)	Post-menopausal (n=18)	Males (n=18)
Age (yr)	25.6 ± 4.7	30.0 ± 8.0	58.1 ± 4.2	38.8 ± 15.8
range	18-35	41-75	51-68	18-68
Smokers (n)	6	4	2	8
Subcutaneous fat (%)	28.9 ± 5.1	27.7 ± 3.4	38.2 ± 3.0	20.7 ± 5.6
Hand volume (ml)	307 ± 25	318 ± 60	350 ± 49	465 ± 66
Bodymass index (kg/m^2)	23.2 ± 3.8	21.0 ± 1.8	24.4 ± 4.2	22.6 ± 2.4
Blood pressure (mmHg)				
Systolic	103.0 ± 10.5	100.0 ± 11.5	115.1 ± 14.3	111.8 ± 14.4
Diastolic	70.5 ± 7.4	72.5 ± 8.0	79.7 ± 9.8	75.6 ± 6.7
Mean	81.3 ± 8.0	81.7 ± 8.6	91.5 ± 10.7	87.7 ± 8.8
Heart rate (beats/min)	67.0 ± 9.9	67.7 ± 10.5	67.5 ± 7.6	64.7 ± 9.9

Furthermore we calculated the mean level during heating, during cooling and during recovery by determining the area under the curve of each test period divided by the duration of that test period.[22]

Statistical analysis was performed by analysis of variance and multiple comparisons according to the method of Tukey. Parameters which showed a skewed distribution (not Gaussian) were first logarithmically transformed. Comparison of the percentual changes was performed by the Kruskal-Wallis test. Results were considered to be significant if $p < 0.05$ (two-sided).

Stepwise regression, with the factor group included, was used to detect an influence of smoking, heart rate, MAP, amount of subcutaneous fat, body mass index and hand volume on the test parameters.

RESULTS

In Table 9.2 the general characteristics of the subjects are shown. Mean age was the highest in the group of postmenopausal women and the lowest in the group of women using oc ($p < 0.001$). The amount of subcutaneous fat was significantly higher in postmenopausal women than in all other groups. In males it was significantly lower in comparison with all female groups (both $p < 0.001$). Hand volume in males was considerably higher than in females ($p < 0.001$). The mean arterial blood pressure in postmenopausal women was significantly higher than in both other groups of women ($p < 0.01$).

Table 9.3 and Figure 9.1 show the results of the finger heating test. Women using oc had the lowest preheating FST (30.5 $\pm$ 1.1°C versus males, 32.2 $\pm$ 1.0°C; $p < 0.01$) and reached the highest maximum FST during heating (women using oc, 39.4 $\pm$ 0.3°C versus males, 39.0 $\pm$ 0.4°C; $p < 0.05$). The percentual increase of FST was significantly different between the groups ($p < 0.001$, median in women using oc 28.9%, in premenopausal women 26.3%, in postmenopusal women 25.3% and in males 21.4%). Preheating LDF was not different between the groups. Both women using oc and premenopausal women reached a higher maximal LDF than males (premenopausal women, 62.3 $\pm$ 10.9 PU versus males, 50.7 $\pm$ 11.0 PU; $p < 0.01$). The percentual increase of LDF was significantly different between the groups as well ($p < 0.01$, median in premenopausal women 198%, in premenopausal women 171%, in postmenopausal women 147% and in males 66%). Both the mean level of FST and LDF during heating showed no significant differences between the groups.

Results of the finger cooling test are shown in Table 9.4 and Figure 9.2. Women using oc had a significantly lower precooling FST than postmenopausal women and males (women using oc, 32.0 $\pm$ 0.9°C versus males, 32.9 $\pm$ 0.8°C; $p < 0.01$). The minimum FST during cooling in women using oc was significantly lower than in

Table 9.3. Finger skin temperature (FST) and laser Doppler flux (LDF in perfusion units, PU) for the four groups (women using oral contraceptives, premenopausal women, postmenopausal women and males) before (preheating value) and during heating (the maximum value, the percentual increase and the mean level during heating). Results are means ± SD.

	Oral contraceptives (oc) (n=18)	Premenopausal (pre) (n=17)	Postmenopausal (pom) (n=18)	Males (m) (n=18)	Comparisons
FST (°C)					
Preheating	30.5 ± 1.1	31.1 ± 1.2	31.3 ± 1.5	32.2 ± 1.0	oc vs m
Maximum	39.4 ± 0.3	39.1 ± 0.4	39.1 ± 0.5	39.0 ± 0.4	oc vs m
Increase (%, median)	28.9	26.3	25.3	21.4	S
Mean	37.4 ± 0.6	37.6 ± 0.6	37.4 ± 0.7	37.8 ± 0.5	NS
LDF (PU)					
Preheating	24.1 ± 15.2	24.2 ± 12.8	25.1 ± 15.7	32.9 ± 11.3	NS
Maximum	62.1 ± 13.8	62.3 ± 10.9	60.7 ± 11.6	50.7 ± 11.0	oc,pre vs m
Increase (%, median)	171	198	147	66	S
Mean	45.0 ± 12.4	46.6 ± 8.0	44.3 ± 11.6	42.2 ± 10.2	NS

Comparisons refer to significant multiple comparisons according the Tukey method. Comparison of the percentual changes was performed by the Kruskal-Wallis test. NS means no significant difference, whereas S means a significant difference.

Table 9.4. Finger skin temperature (FST) and laser Doppler flux (LDF in perfusion units, PU) before (precooling value), during (the minimum value, the percentual decrease and the mean level during cooling) and after cooling (the mean level during the recovery period) for the four groups: women using oral contraceptives, premenopausal women, postmenopausal women and males. Results are means ± SD.

	Oral contraceptives (oc) (n=18)	Premenopausal (pre) (n=17)	Postmenopausal (pom) (n=18)	Males (m) (n=18)	Comparisons
FST (°C)					
Precooling	32.0 ± 0.9	32.2 ± 0.9	32.8 ± 0.8	32.9 ± 0.8	oc vs pom & m
Mininum	20.9 ± 0.7	21.4 ± 1.0	22.3 ± 1.5	23.2 ± 2.3	oc vs pom & m & pre vs m
Decrease (%, median)	34.2	34.0	32.3	30.7	S
Mean during cooling	25.5 ± 1.7	25.9 ± 1.0	26.8 ± 1.1	27.1 ± 1.4	oc vs pom & m & pre vs m
Mean during recovery	22.0 ± 2.5	23.5 ± 3.0	24.5 ± 3.5	26.5 ± 4.4	oc vs m
LDF (PU)					
Precooling	33.8 ± 15.5	29.6 ± 11.0	35.9 ± 14.5	37.6 ± 11.1	NS
Minimum	8.1 ± 7.6	8.3 ± 7.1	9.8 ± 6.9	19.5 ± 18.4	oc &pre vs m
Decrease (%, median)	83	79	77	61	NS
Mean during cooling	14.4 ± 9.2	12.5 ± 5.9	16.7 ± 9.0	24.4 ± 14.8	oc & pre vs m
Mean during recovery	11.1 ± 13.4	12.0 ± 11.0	16.0 ± 14.9	25.4 ± 17.2	oc & pre vs m

Comparisons refer to significant multiple comparisons according the Tukey method. Comparison of the percentual changes was performed by the Kruskal-Wallis test. NS means no significant difference, whereas S means a significant difference.

postmenopausal women and males, as was the minimum FST in premenopausal women compared to males (women using oc, 20.9 ± 0.7°C versus males, 23.2 ± 2.3°C; $p<0.001$). The same differences between the groups were observed for the

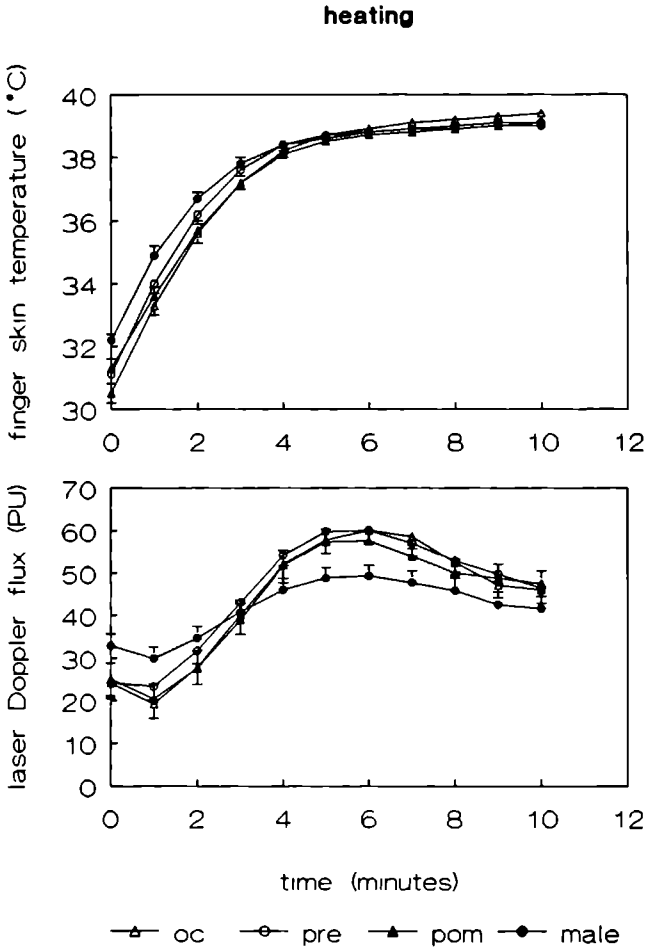

Figure 9.1. Finger skin temperature (upper panel) and laser Doppler flux (in perfusion units, PU, lower panel) during 10 min of immersion of the hand in a 45°C water bath in the four groups: women using oral contraceptives (oc), premenopausal women (pre), postmenopausal women (pom) and males. Means and SEM are given; when the SEM can not be distinguished it is within the symbol.

mean level of FST during cooling (women using oc, 25.2 ± 1.6°C versus males, 27.1 ± 1.4°C; $p<0.001$). The percentual decrease was significantly different between the groups ($p<0.01$, median in males 30.7%, in postmenopausal women 32.3%, in

premenopausal women 34.0% and in women using oc 34.2%). During recovery, the mean level of FST was the highest in males (males, 26.5 ± 4.4°C versus women using oc, 22.0 ± 2.5°C; <0.01).

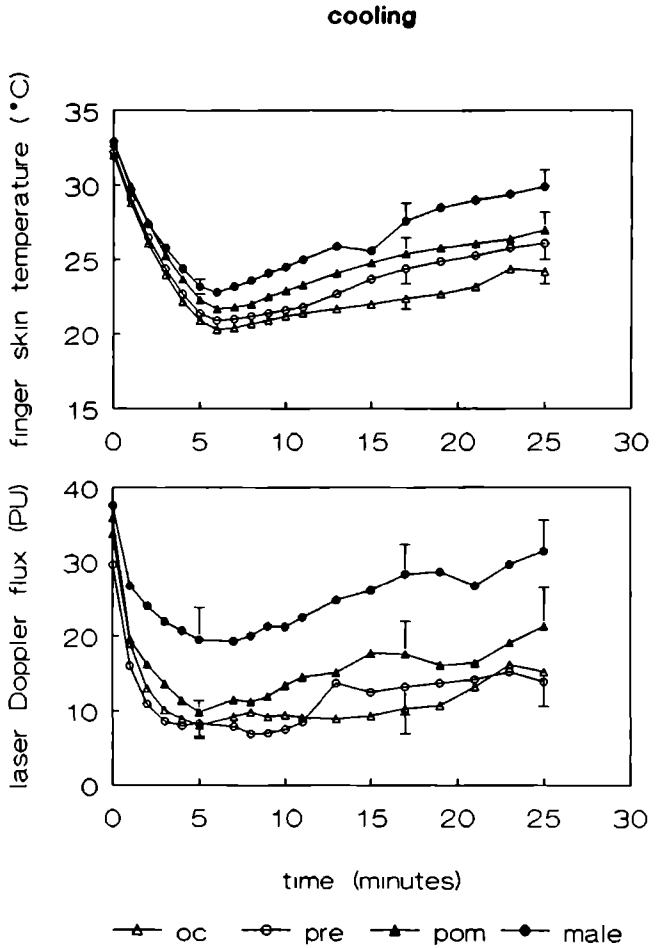

Figure 9.2. Finger skin temperature (upper panel) and laser Doppler flux (in perfusion units, PU, lower panel) during immersion of the hand in a 15°C water bath for the first 5 min and a recovery period of 20 min in the four groups: women using oral contraceptives (oc), premenopausal women (pre), postmenopausal women (pom) and males. Means and SEM (at three points) are given.

No significant differences in precooling LDF were seen between the groups (premenopausal women, 29.6 ± 11.0 PU versus males, 37.6 ± 11.1 PU). The minimum LDF during cooling was significantly lower in women using oc and in premenopausal women than in males (women using oc, 8.1 ± 7.6 PU versus males,

19.5 ± 18.4 PU; $p<0.05$). The mean level of LDF during cooling showed the same results (premenopausal women, 12.5 ± 5.9 PU versus males, 24.4 ± 14.8 PU; $p<0.01$). The percentual decrease in LDF was not significantly different between the groups. The mean level of LDF during recovery was the highest in males (25.4 ± 17.2 PU versus women using oc, 11.1 ± 13.4 PU; $p<0.01$).

Stepwise regression with the groups included as factor showed a significant influence of the group and not of biological factors (age, amount of subcutaneous fat, hand volume and body mass index) or hemodynamic parameters (MAP and heart rate).

DISCUSSION

This study shows that skin vascular reactivity to heat and cold challenge is different between males and females and that skin vascular reactivity to a cold challenge is different between females with a different hormonal status as well. Women who use oc and premenopausal women showed the most pronounced changes in skin blood flow during both heating and cooling and recovered slower after cooling. The vascular reactivity in postmenopausal women was intermediate between the males and the two other groups of women.

The groups were not matched and differed in age, amount of subcutaneous fat, hand volume, body mass index or hemodynamic parameters (MAP and heart rate). However, these factors are not likely to account for the observed differences between the groups, as they were all found not to have influenced the results.

In a previous study we have shown that vascular reactivity towards cold is less in case of a low baseline skin blood flow.[23] Therefore we have used a 30°C water bath before provocation to obtain comparable pre-test values in the four groups. Despite this, women who used oc exhibited both a lower preheating and a lower precooling FST compared with males. In order to correct for this, we also calculated percentual changes.

Bollinger et al[6] determined FST and finger blood flow by venous occlusion plethysmography in healthy postmenopausal and premenopausal women and males. They found nearly no difference in finger blood flow at rest between postmenopausal women and males. In both groups finger blood flow was significantly higher than in premenopausal women, although no significant differences in FST were observed. The prolonged recovery time after direct cooling in the premenopausal group is in correspondence with our findings. Tooke et al[13] investigated the influence of oc on finger skin temperature, nailfold capillary pressure and finger blood flow, the latter measured by venous occlusion plethysmography. In contrast with our results they found significantly higher baseline finger blood flow values and capillary pressure in

women using oc when compared with premenopausal women without oc, whereas FST was not significantly different. A lower baseline LDF[7] and a lower baseline FST[9] were observed in healthy females compared with healthy males. In both of these studies no further subdivision in the group of females was made.

In the present study, women using synthetic, exogenous hormones showed the most pronounced vasodilation as well as vasoconstriction during respectively heating and cooling, and in the recovery period after cooling vasoconstriction continued. Thus, vascular reactivity towards both heat and cold challenge seems to be enhanced by exogenous synthetic estrogens and progestagens. Premenopausal women, investigated in a phase with low endogenous sex hormone levels, showed a vascular reactivity comparable with women using oc. How to explain these apparently contrasting findings? Endogenous and exogenous hormones may exert opposite effects on the peripheral skin circulation, as it is not well documented whether natural and synthetic oestrogens exhibit the same effect on the same target.[18] However, a more likely explanation of the findings is that although estradiol and progesterone were only present in low concentrations in premenopausal women at the time of menstruation, the presence of these hormones in fluctuating levels during the menstrual cycle accounted for a state of high vascular reactivity. In accordance with this hypothesis postmenopausal women, who also have low concentrations of endogenous female sex hormones, showed a pattern different from that of the other two female groups, being more in line with the males. This latter group showed less vasodilation during heating and less vasoconstriction during cooling and recovered more rapidly after cooling. The findings in the postmenopausal and male group could be ascribed to the absence (at least in concentrations high enough to exert any influence) of the female sex hormones which would have enhanced vascular reactivity or, alternatively, to the presence of androgens in (relatively) high concentrations, which might depress vascular reactivity. However, rather than the presence or absence of a specific sex hormone, the ratio of 17ß-estradiol, progesterone and testosterone may be the most important, as previously suggested.[14] LH and FSH are not likely to influence (e.g. depress) vascular reactivity, as the postmenopausal females (high levels) do not differ from the males (low levels).

The precise mechanism by which sex hormones are able to modulate skin blood flow is still unknown. They could exert a direct influence on the blood vessel wall. In the endothelial and vascular smooth muscle cells of animals estrogen receptors have been demonstrated.[24] Sex hormones could also influence the production of vasoactive substances produced by the endothelial or by the blood cells.[18] Besides, sex hormones could indirectly influence regulatory mechanisms of the peripheral circulation. An influence on the sympathetic nervous system has been described[17,18,25] The sympathetic nervous system releases catecholamines that stimulate vasoconstrictive α-adrenoceptors

and vasodilating $ß_2$-adrenoceptors. 17ß-Estradiol modulates the response to sympathetic stimuli by enhancing vasoconstriction by mechanisms not yet fully understood. Upregulation of α-adrenoceptors may play a part in this complicated process.[26 27 28] Finally, sex hormones could exert another, more systemic influence. For example, oc stimulate the renin-angiotensin-aldosterone system,[29 30] thereby increasing peripheral resistance. Also fibrinogen and hemorheological indices such as viscosity show a variation within the menstrual cycle and are elevated in women using oc.[31]

Although the precise mechanisms still remain obscure, from the present study we conclude that the hormonal status exerts significant effects on finger skin perfusion. Synthethic as well as endogenous estrogens and/or progesterone cause enhanced skin vascular reactivity, whereas testosterone may depress skin vascular reactivity. Most likely FSH and LH have no important modulating effect.

As a consequence of these observed differences in finger skin vascular reactivity between males and females and between females with a different hormonal status, in future studies in which finger skin perfusion is measured the hormonal status of the subjects should be taken into account.

REFERENCES

1 Gifford RW, Hines EA. Raynaud's disease among women and girls. Circulation 1957;16:1012-21.
2 de Trafford JC, Lafferty K, Potter CE, Roberts VC, Cotton LT. An epidemiological survey of Raynaud's phenomenon. Eur J Vasc Surg 1988;2:176-80.
3 Holti G. Raynaud's phenomenon. Adv Microcirc 1982;10:1-16.
4 Wollersheim H, Thien Th. The diagnostic value of clinical signs and symptoms in patients with Raynaud's phenomenon. A cross-sectional study. Neth J Med 1990;37:171-82.
5 Jarret PEM. Raynaud's disease and oral contraceptives. Br Med J 1976;2:477.
6 Bollinger A, Schlumpf M. Finger blood flow in healthy subjects of different age and sex and in patients with Raynaud's disease. Acta Chir Scand 1974;suppl:42-7.
7 Cooke JP, Creager MA, Osmundson PJ, Shepherd JT. Sex differences in control of cutaneous blood flow. Circulation 1990;82:1607-15.
8 van de Wal HJCM, Wijn PFF, van Lier HJJ, Kneepkens WGHJ, Skotnicki SH. Noninvasive assessment of vasospasm in patients with primary Raynaud's phenomenon. Angiology 1987;38:315-32.
9 Webb RC, Rusch NJ, Vanhoutte PM. Influence of sex difference and oral contraceptives on forearm reactive hyperemia. Blood Vess 1981;18:161-70.
10 Wouda AA. Raynaud's phenomenon. Acta Med Scand 1977;201:519-23.
11 Keates JS, FitzGerald DE. Effect of oral contraceptives on the peripheral vasculature of women. Bibl Anat 1975;13:356-7.
12 Lehtovirta P. Peripheral haemodynamic effects of combined estrogen/progestogen oral contraceptives. J Obst Gyn Br Commonwealth 1974;81:526-34.
13 Tooke JE, Tindall H, McNicol GP. The influence of a combined contraceptive pill and menstrual cycle phase on digital microvascular haemodynamics. Clin Sci 1981;61:91-5.
14 Bartelink ML, Wollersheim H, Theeuwes A, van Duren D, Thien Th. Changes in skin blood flow during the menstrual cycle. Clin Sci 1990;78:527-32.

15 Lafferty K, de Trafford JC, Potter C, Roberts VC, Cotton LT. Reflex vascular responses in the finger to contralateral thermal stimuli during the normal menstrual cycle: a hormonal basis to Raynaud's phenomenon. Clin Sci 1985;68:639-45.
16 Terregino CA, Seibold JR. Influence of the menstrual cycle on Raynaud's phenomenon and on cold tolerance in normal women. Angiology 1985;36:88-95.
17 Altura BM. Sex and estrogens and responsiveness of terminal arterioles to neurohypophyseal hormones and catecholamines. J Pharmacol Exp Ther 1975;193:403-21.
18 Altura BM, Altura BT. Influence of sex hormones, oral contraceptives and pregnancy on vascular muscle and its reactivity. In: Carrier O, Shibata S, eds. Factors influencing vascular reactivity. Tokyo: Igaku-Shoin, 1977:221-54.
19 Elstein M, Morris SE, Groom GV, Jenner DA, Scarisbrick JJ, Cameron EHD. Studies on low-dose oral contraceptives: Cervical mucus and plasmahormone changes in relation to circulating norgestrel and 17α-ethinylestradiol concentrations. Fertil Steril 1976;27:892-9.
20 Cleophas TJM, Fennis JFM, van 't Laar A. Finger temperature after a finger cooling test. Influence of airtemperature and cigarette smoking. J Appl Physiol 1982;52:1167-71.
21 Durnin JVGA, Womersly J. Body fat assessed from total body density and its estimation from skinfold thickness. Br J Nutr 1974;32:77-9.
22 Wollersheim H, Thien Th, Fennis J, van Elteren Ph, van 't Laar A. Double-blind, placebo-controlled study of prazosin in Raynaud's phenomenon. Clin Pharm Ther 1986;40:219-25.
23 Wollersheim H, Droste H, Reyenga J, Thien Th. Laser Doppler evaluation of skin vasomotor reflexes during sympathetic stimulation in normals and in patients with primary Raynaud's phenomenon. Int J Microcirc Clin Exp 1991;10:33-42.
24 McGill HC, Sheridan PJ. Nuclear uptake of sex steroid hormones in the cardiovascular system of the baboon. Circ Res 1981;48:238-44.
25 Levine JD, Taiwo YO. ß-Estradiol induced catecholamine-sensitive hyperalgesia: a contribution to pain in Raynaud's phenomenon. Brain Res 1989;487:143-7.
26 Colucci WS, Gimbrone MA, McLaughlin MK, Halpern W, Alexander RW. Increased vascular catecholamine sensitivity and α-adrenergic receptor affinity in female and estrogen-treated male rats. Circ Res 1982;50:805-11.
27 Jones SB, Bylund DB, Bieser CA, Sherim WO, Beyer JA, Carr GW. α-Adrenergic receptor binding in human platelets: alterations during the menstrual cycle. Clin Pharmacol Ther 1983;34:90-6.
28 Mishra N, Hamilton CA, Jones CR, Leslie C, Reid JL. α-Adrenoceptor changes after estrogen treatment in platelets and other tissues in female rabbits. Clin Sci 1985;69:235-8.
29 Skinner SL, Lumbers ER, Symonds EM. Alteration by oral contraceptives of normal menstrual changes in plasma renin activity, concentration and substrate. Clin Sci 1969;36:67-76.
30 Walters WAW, Lim YL. Hemodynamic changes in women taking oral contraceptives. J Obst Gyn Br Commonwealth 1976;77:1007-12.
31 Buchan PC, Macdonald HN. Altered haemorheology in oral-contraceptive users. Br Med J 1980;978-9.

CHAPTER 10

EFFECT OF SINGLE-DOSE 17β-ESTRADIOL AND PROGESTERONE ADMINISTRATION ON FINGER SKIN CIRCULATION IN FEMALE HEALTHY SUBJECTS AND RAYNAUD PATIENTS

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Submitted

ABSTRACT

The influence of sex, menstrual cycle, oral contraceptives, pregnancy and menopause on skin perfusion in healthy subjects and in Raynaud patients suggests a role of female sex hormones. However, no clear relationship between skin blood flow and levels of circulating estrogens or progestagens has been found yet. Aim of the present study was to investigate the effect of orally administered 17β-estradiol and progesterone on finger skin blood flow before and during heat and cold challenge in 17 healthy normotensive female volunteers and in 12 female Raynaud patients. In each subject a standardized finger heating (45 °C water bath, 10 min) and cooling test (15 °C water bath, 5 min and 20 min recovery) was performed twice on the second (or third) day of two consecutive menstrual cycles. 17β-Estradiol (9 mg) and progesterone (300 mg) were administered in a randomized order in a double blind placebo-controlled design. Both doses resulted in (high) physiological levels. During the experiment, fingertip skin temperature (FST) and laser Doppler flux (LDF) were measured. There were no significant differences in test results after placebo and after progesterone administration. Although values of FST and LDF after 17β-estradiol administration tended to be higher, only precooling values in the healthy subjects reached significance (FST with placebo and with estradiol (mean ± SD), respectively, 32.7 ± 1.0 and 33.1 ± 0.8 °C; LDF with placebo and with estradiol, 33.6 ± 11.7 and 42.2 ± 9.5 perfusion units; both $p < 0.05$). In this study, short term administration of female sex hormones had only minor influence on finger skin circulation, both in control subjects and in Raynaud patients.

INTRODUCTION

Animal studies show that estrogens and progestagens exert effects on the peripheral circulation.[1] Several observations strongly suggest that estrogens and progestagens are also able to influence peripheral skin circulation in humans. In general, the differences in skin blood flow between men and women after menarche and before menopause[2,3] are explained this way. Finger skin perfusion and its response to a local cold challenge were demonstrated to variate during the menstrual cycle with the lowest perfusion in the mid-luteal phase (high levels of both 17ß-estradiol and progesterone) and the highest perfusion pre-ovulatory (high levels of 17ß-estradiol).[4] Oral contraceptives[5] and pregnancy[6] have also been shown to influence peripheral skin circulation.

Raynaud's phenomenon (RP), a vasospastic syndrome with acroteric discolourations provoked by cold or emotions, is more common in women.[7] Furthermore, the complaints of RP are influenced by the phase of the menstrual cycle, the use of oral contraceptives, and by pregnancy and menopause.[8]

However, no relationship with the levels of circulating estrogens or progestagens has been found up to now. The aim of the present study was to investigate the effect of orally administered natural 17ß-estradiol and progesterone on finger skin blood flow before and during standardized heat and cold challenge. Because a possible influence of sex hormones might be more pronounced in subjects with an already impaired finger blood flow, both female healthy subjects and female Raynaud patients were studied.

SUBJECTS AND METHODS

Subjects

We selected 18 healthy normotensive female volunteers without any sign or symptom of a vascular disease who did not use any vasoactive medication. None had complaints of discolorations of fingers or toes. The second group consisted of 13 female patients with primary RP (according to the criteria of Allen and Brown[9], supplemented with negative clinical[8] and immunological[10] signs of an underlying disease), who did not use medication for their complaints. They all had regular menstrual cycles (between 26 and 32 days) and had not used oral contraceptives for at least 6 months. The study protocol was approved by the local ethical committee and all subjects gave written informed consent.

Methods

The present study was carried out in the early follicular phase of the menstrual cycle when levels of endogenous sex hormones are low. In each subject a standardized finger heating and cooling test was performed twice on the second (or third) day of two consecutive menstrual cycles. On each test day, the first test was always performed after oral placebo administration (single blind) and the second test after randomized oral 17ß-estradiol or progesterone administration (double blind). The subjects were instructed to take the placebo tablets 90 min prior to arrival in the hospital. After the first test was performed, they took either 9 mg 17ß-estradiol (tablets of 1.5 mg micronized 17ß-estradiol, Organon, Oss, The Netherlands) or 300 mg progesterone (tablets of 100 mg micronized progesterone: Progestan, Organon, Oss, The Netherlands). After another 90 min, the second test was started. The dose of each hormone was chosen after pilot experiments during which frequent venous blood samples were taken (with an interval of 20 min during 3 hr). Aim was to obtain high, but still physiological levels of both hormones during the experiments. In the normal menstrual cycle high blood levels of 17ß-estradiol are seen in the luteal phase (between 220 and 1000 pmol/l); the pre-ovulatory peak may reach values up to 1800

pmol/l. The luteal progesterone level varies between 19 and 120 nmol/l.

Factors influencing peripheral circulation were eliminated by asking all subjects to refrain from respectively smoking for 24 hr,[11] alcohol- and caffeine-containing beverages for 12 hr, and not to eat for at least 1 hr prior to the tests. After each first test they were allowed to have a light meal. The tests were performed in a quiet climatized room with a constant ambient temperature (mean ± SD) of 24.5 ± 1°C and a humidity of 59 ± 0.9%. All subjects were lightly dressed and rested supine with their arms supported on heart level.

Before the first test, before the second test (90 min after intake) and after the second test (180 min after intake), blood was sampled by venepuncture of the antecubital vein for determination of serum levels of 17ß-estradiol, progesterone, luteinizing hormone (LH) and follicle stimulating hormone (FSH). The samples were frozen (-20°C) and the sex hormone levels were determined afterwards by specific radioimmunoassays,[12] whereas the pituitary hormones were measured by applying specific immunoradiometric assays (Medgenix, Fleurus, Belgium), as described previously.[13] After the experiments, the available hormonal data were submitted to an independent, experienced observer, who verified the menstrual phase of the cycle. Prior to the study minimal values that should be exceeded were defined as follows: 500 pmol/l after 17ß-estradiol administration and 10 nmol/l after progesterone administration.

During the test the rectal temperature was recorded continuously (in °C, Yellowspring probe, type YSI 401 combined with a Hewlett Packard datalogger). The amount of subcutaneous fat (% of total body mass) was determined by measuring skinfold thickness at four standardized sites of the body (in mm), after which the corresponding percentage of subcutaneous fat according to the age and sex was read from a table.[14] The hand volume (in ml) was determined by the increase in volume after immersion of the hand up to the wrist in a definite amount of water. Finger circumference (third finger) was measured (in cm), and length and weight were determined to assess the body mass index.

After an acclimatization period of at least 20 minutes systolic and diastolic blood pressure were measured at the left arm by an Arteriosonde (in mmHg; Roche Medical Electronics Inc., Oranjeburg, NJ, USA). The heart rate (in beats per minute) was calculated from 10 RR-intervals of an electrocardiogram-strip.

Forearm muscle blood flow (FBF) was measured at the left forearm by strain gauge venous occlusion plethysmography (in ml/100 ml/min; Loosco BVP 96, Hoekloos, Amsterdam, The Netherlands). A wrist cuff was inflated to a suprasystolic value to exclude the hand circulation.[15] On every time point the average of three subsequent measurements was taken.

Finger skin perfusion was quantified by laser Doppler fluxmetry (LDF) and determination of finger skin temperature (FST). LDF was measured with an unheated

probe on the third volar fingertip of the right hand (in perfusion units (PU); Periflux Pf-ld, Perimed, Stockholm, Sweden). The gain was adjusted to 3, the cutoff frequency to 12 kHz and the time constant to 3 sec. LDF was continuously registered on a chart recorder. Flux zero calibration was performed by readjusting the pen of the recorder to zero, when the probe was fixed to a white, non-moving surface. Once a week a calibration with 25%-calibration fluid (Periflux motility standard, Perimed) was performed. LDF values were calculated afterwards as the mean of a one minute period. The biological zero was determined by arresting the circulation of the third finger during 5 min by a cuff inflated to a suprasystolic value. The resulting LDF signal was readjusted to zero. To prevent a variation in LDF between the first and the second test due to a change in the position of the probe, the probe holder remained fixed on the finger in between both tests.

FST (in °C) was measured at the volar side of the intermediate phalanx of the third finger (Thermocouple, Ellab Instruments, Copenhagen, Denmark). The sensor part was isolated on the outside.

Before heating and cooling the gloved right hand was immersed in a 30°C water bath during 10 min. Preheating and precooling LDF values were obtained by the average of three 1-min values during the last 5 min. The preheating and precooling FST were the values just before respectively heating and cooling.

Heating was performed by immersing the gloved right hand in water of 45°C during 10 min and cooling in water of 15°C during 5 min. During these procedures the momentary FST was registered and the averaged mean LDF of that minute was added afterwards. A subsequent recovery period of 20 min followed after cooling with the hand exposed to room temperature. During recovery LDF and FST were obtained every min in the first 6 min and every 2 min afterwards.

Data analysis and statistics

Unless stated otherwise, results are shown as means ± SD. The mean arterial pressure (MAP; in mmHg) was calculated from the formula MAP=(SBP+2DBP)/3. Forearm vascular resistance (FVR; in arbitrary units) was obtained by dividing MAP by FBF. Laser Doppler resistance in the skin (in mmHg/PU) was calculated by dividing MAP by baseline LDF.[16] The body mass index was expressed as kg/m^2. The following parameters for both FST and LDF were used in the analysis: the preheating value, the maximum value during heating, the precooling value, the minimum value and the percentual decrease during cooling. Furthermore, we calculated the mean level during heating, during cooling, and during recovery by determining the area under the curve of each test period divided by the length in min of the test period.

The differences between the placebo test and the hormone tests were used in the statistical analysis, which was performed by the paired Student t test or, if these

differences were not normally distributed, by the signed rank test. Pearson correlation coefficients were calculated between the difference in hormone levels before and during the test and the changes that took place in FST or in LDF. Results were considered to be significant if the p value was less than 0.05 (two-sided).

RESULTS

Hormone levels

Table 10.1 shows serum levels of 17ß-estradiol, progesterone, FSH and LH, before and after administration. All but one subject in the Raynaud group (with high levels of 17ß-estradiol before administration of this hormone) were in the menstrual phase. Another subject in this group did not reach a sufficiently high level of progesterone after administration of this hormone (4.7 nmol/l and 11.0 nmol/l after 90 and 180 min, respectively). Therefore, in the comparison of either the 17ß-estradiol or the progesterone test 12 subjects were included. In the healthy subjects, 17 subjects were analyzed: in one female we were not able to reach sufficient levels of both sex hormones (17ß-estradiol: 190 and 220 pmol/l and progesterone: 3.4 and 4.2 nmol/l after 90 and 180 min, respectively). There were no significant differences between the groups regarding the baseline hormone levels or the levels reached after administration of both sex hormones.

Table 10.1. Hormone levels at different time points in both groups.

	Healthy subjects (n=17)	Raynaud patients (n=12)
17ß-Estradiol (pmol/l)		
baseline	213 ± 70	256 ± 37
90 min after intake	923 ± 271	931 ± 248
180 min after intake	984 ± 237	962 ± 318
Progesterone (nmol/l)	1.6 ± 0.5	3.0 ± 3.3
Luteinizing Hormone (IU/l)	3.2 ± 1.5	5.8 ± 1.8
Follicle Stimulating Hormone (IU/l)	6.1 ± 2.7	7.9 ± 4.1
Progesterone (nmol/l)		
baseline	2.1 ± 0.9	2.0 ± 0.5
90 min after intake	170 ± 351	87 ± 132
180 min after intake	63 ± 87	36 ± 37
17ß-Estradiol (pmol/l)	226 ± 85	239 ± 91
Luteinizing Hormone (IU/l)	3.8 ± 1.6	5.0 ± 1.2
Follicle Stimulating Hormone (IU/l)	5.9 ± 2.6	8.7 ± 5.5

Means ± SD are given. IU means international units.

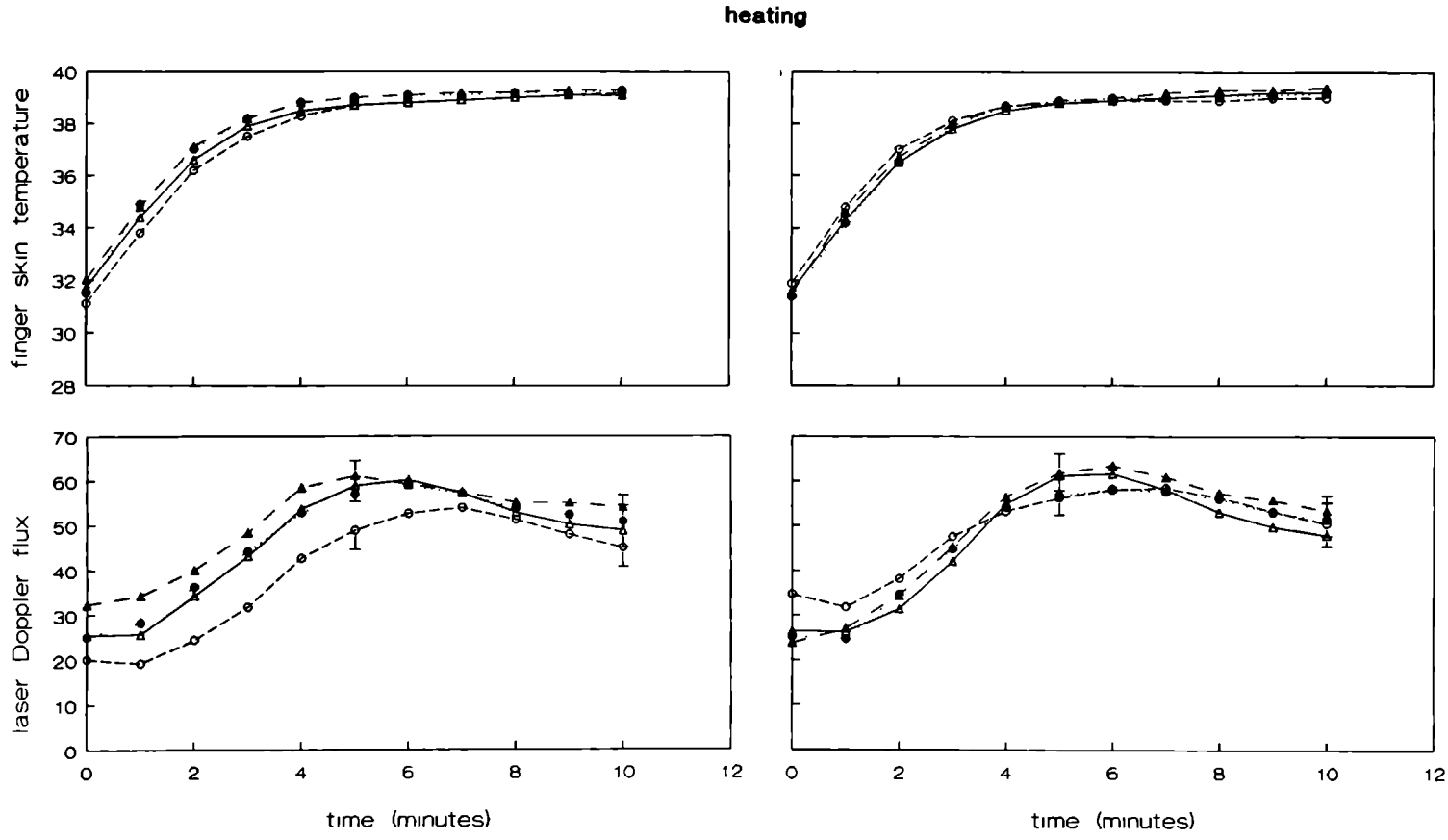

Figure 10.1. Mean finger skin temperature (in °C) and laser Doppler flux (in perfusion units, PU) during finger heating with placebo (open symbols) and with 17β-estradiol (closed symbols, left panel), and with placebo (open symbols) and with progesterone (closed symbols, right panel) in healthy subjects (triangles) and in Raynaud patients (dots). SEM is given at 5 and 10 min.

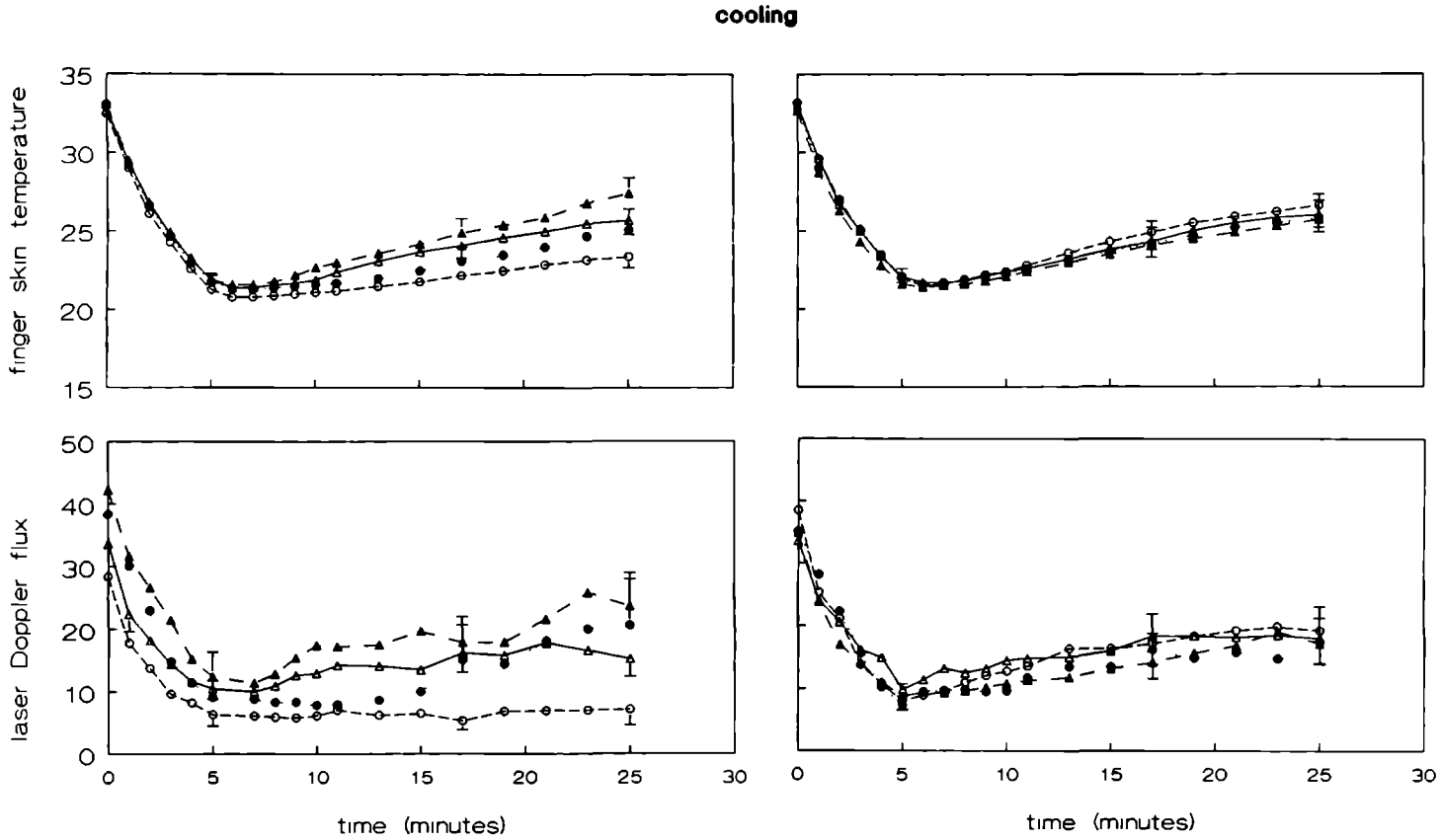

Figure 10.2. Mean finger skin temperature (in °C) and laser Doppler flux (in perfusion units, PU) during finger cooling with placebo (open symbols) and with 17β-estradiol (closed symbols, left panel), and with placebo (open symbols) and with progesterone (closed symbols, right panel) in healthy subjects (triangles) and in Raynaud patients (dots). SEM is given at 5, 17 and 25 min.

General characteristics

There were no major differences between the healthy subjects and the Raynaud patients with regard to respectively age (29.8 ± 7.2 and 35.3 ± 5.7 yr), body mass index (22.2 ± 1.7 and 21.7 ± 2.6 kg/m^2), amount of subcutaneous body fat (28.6 ± 4.4 and 29.1 ± 5.2%), hand volume (307 ± 37 and 313 ± 23 ml), and finger circumference (6.2 ± 0.3 and 6.2 ± 0.4 cm).

Table 10.2. Hemodynamic baseline parameters before 17β-estradiol administration (placebo), the differences (represented by the symbol Δ) between the test after placebo and the test after 17β-estradiol administration, and the differences between the test after placebo and the test after progesterone administration.

Healthy subj. (n=17)	Placebo	Δ (Placebo-17ß-Estradiol)	Δ (Placebo-Progesterone)
Blood pressure (mmHg)			
Systolic	101.4 ± 7.7	0.8 ± 5.8	0.8 ± 6.3
Diastolic	62.8 ± 5.7	0.3 ± 3.9	-0.8 ± 4.2
Mean	75.6 ± 5.8	0.5 ± 3.7	-0.3 ± 4.2
Heart rate (beats/min)	66.5 ± 9.8	-1.3 ± 7.6	0.3 ± 8.5
Forearm Blood Flow (ml/100ml/min)	2.3 ± 1.0	-0.2 ± 0.9	-0.4 ± 1.0
Forearm Vascular Resistance (AU)	37.7 ± 14.2	3.5 ± 14.1	6.0 ± 24.4
Raynaud pat. (n=12)	**Placebo**	**Δ (Placebo-17ß-Estradiol)**	**Δ (Placebo-Progesterone)**
Blood pressure (mmHg)			
Systolic	104.3 ± 8.0	3.2 ± 6.5	1.3 ± 6.1
Diastolic	70.1 ± 7.7	6.8 ± 5.1	3.5 ± 6.8
Mean	81.5 ± 7.4	5.6 ± 4.3	2.8 ± 5.7
Heart rate (beats/min)	65.3 ± 7.1	-6.7 ± 7.9	-0.8 ± 6.3
Forearm Blood Flow (ml/100ml/min)	2.0 ± 0.5	-1.0 ± 1.5	-0.1 ± 1.4
Forearm Vascular Resistance (AU)	44.3 ± 13.2	11.2 ± 17.4	5.5 ± 13.6

Means ± SD are given. AU means arbitrary units.

Baseline hemodynamic parameters

Table 10.2 shows that there were no changes in the hemodynamic parameters in the healthy subjects after the administration of both 17ß-estradiol and progesterone. In the

Raynaud patients progesterone induced no significant hemodynamic changes. After 17ß-estradiol systolic blood pressure did not decrease significantly, but the decrease in diastolic blood pressure was significant (from 70.1 ± 7.7 to 63.3 ± 8.1 mmHg, $p<0.01$). Also the MAP decreased significantly from 81.5 ± 7.4 to 75.9 ± 7.3 mmHg ($p<0.01$). Heart rate increased significantly after 17ß-estradiol administration from 65.3 ± 7.1 to 72.0 ± 8.3 beats/min ($p<0.05$). Although a rise in FBF was seen of about 50%, it did not reach significance ($p=0.05$). As a result, FVR decreased from 44 ± 13 to 33 ± 17 AU ($p=0.05$).

Table 10.3. Skin circulation parameters before 17ß-estradiol administration (placebo), the differences (represented by the symbol Δ) between the test after placebo and the test after 17ß-estradiol administration, and the differences between the test after placebo and the test after progesterone administration in the healthy subjects.

Healthy subjects (n=17)	Placebo	Δ (Placebo-17ß-Estradiol)	Δ (Placebo-Progesterone)
Rectal temperature (°C)	36.8 ± 0.2	-0.1 ± 0.2	0.0 ± 0.2
Finger Heating Test			
FST (°C)			
preheating	31.7 ± 1.0	-0.3 ± 1.1	0.1 ± 1.2
maximum	39.2 ± 0.4	-0.2 ± 0.5	-0.2 ± 0.5
mean during heating	37.8 ± 0.4	-0.3 ± 0.6	-0.1 ± 0.6
LDF (PU)			
preheating	25.5 ± 10.8	-6.8 ± 13.3	2.5 ± 15.4
maximum	61.5 ± 16.6	-1.6 ± 13.4	-1.6 ± 17.3
mean during heating	47.3 ± 12.3	-3.9 ± 11.4	-2.6 ± 15.0
Finger Cooling Test			
FST (°C)			
precooling	32.7 ± 1.0	-0.4 ± 0.8	0.3 ± 0.9
end of cooling	21.9 ± 1.3	-0.1 ± 1.5	0.5 ± 1.4
mean during cooling	26.2 ± 1.0	-0.1 ± 1.2	0.6 ± 1.4
12 min recovery	24.1 ± 3.4	-0.7 ± 4.0	0.2 ± 3.1
mean recovery	23.5 ± 2.7	-0.7 ± 3.1	0.3 ± 2.4
LDF (PU)			
precooling	33.6 ± 11.7	-8.6 ± 9.5	-1.3 ± 13.0
end of cooling	8.9 ± 5.1	-2.1 ± 7.4	0.9 ± 6.7
mean during cooling	16.6 ± 7.1	-7.7 ± 10.8	0.8 ± 8.2
12 min recovery	16.2 ± 12.6	-1.8 ± 18.0	4.3 ± 14.7
mean recovery	12.8 ± 8.3	-4.8 ± 14.7	-0.1 ± 6.4

Means ± SD are given. PU means perfusion units.

Rectal temperature

Rectal body temperature (Table 10.3) did not change significantly after progesterone. In the healthy subjects, but not in the Raynaud patients, rectal temperature was 0.1

± 0.2°C higher after 17ß-estradiol ($p<0.05$). Although this difference reached significance, it does not seem relevant.

Finger skin circulation (Tables 10.3 and 10.4, Figures 10.1 and 10.2)

Preheating LDF in the Raynaud group was significantly lower after progesterone administration (the difference was 9.6 ± 11.6 PU, $p<0.05$). None of the parameters during finger heating were significantly influenced by administration of either hormone.

Table 10.4. Skin circulation parameters before 17ß-estradiol administration (placebo), the differences (represented by the symbol Δ) between the test after placebo and the test after 17ß-estradiol administration, and the differences between the test after placebo and the test after progesterone administration in the Raynaud patients.

Raynaud patients (n=12)	Placebo	Δ (Placebo-17ß-Estradiol)	Δ (Placebo-Progesterone)
Rectal temperature (°C)	36.8 ± 0.3	-0.1 ± 0.2	-0.1 ± 0.3
Finger Heating Test			
FST (°C)			
preheating	31.1 ± 2.0	-0.4 ± 2.1	0.5 ± 1.5
maximum	39.3 ± 0.5	-0.1 ± 0.5	-0.2 ± 0.4
mean during heating	37.5 ± 0.2	-0.5 ± 0.7	0.1 ± 0.5
LDF (PU)			
preheating	20.1 ± 16.1	-5.0 ± 20.0	9.6 ± 11.6
maximum	55.8 ± 13.3	-3.8 ± 13.7	-1.2 ± 10.8
mean during heating	40.5 ± 12.0	-7.3 ± 14.8	1.8 ± 12.0
Finger Cooling Test			
FST (°C)			
precooling	32.5 ± 0.7	-0.6 ± 1.0	0.0 ± 0.9
end of cooling	21.3 ± 0.7	-0.5 ± 1.2	-0.1 ± 0.9
mean during cooling	25.7 ± 0.8	-0.4 ± 1.2	0.1 ± 1.1
12 min recovery	22.2 ± 1.1	-0.9 ± 2.9	0.7 ± 4.2
mean recovery	21.9 ± 1.0	-0.8 ± 2.3	0.5 ± 3.4
LDF (PU)			
precooling	28.4 ± 18.6	-10.0 ± 17.3	3.4 ± 11.4
end of cooling	6.3 ± 6.2	-2.8 ± 9.2	0.7 ± 2.1
mean during cooling	13.3 ± 9.3	-7.3 ± 11.8	-0.9 ± 5.5
12 min recovery	5.3 ± 4.8	-9.8 ± 18.1	1.0 ± 23.0
mean recovery	6.4 ± 4.9	-6.1 ± 13.8	-0.8 ± 10.6

Means ± SD are given. PU means perfusion units.

Only in the healthy subjects, precooling FST, precooling LDF and the mean LDF during cooling were significantly higher after 17ß-estradiol (the differences were 0.4 ± 0.8°C, 8.6 ± 9.5 PU and 7.7 ± 10.8 PU respectively, all $p<0.05$). Laser

Doppler resistance did also not show significant differences after placebo and after 17ß-estradiol, nor in the Raynaud patients (8.5 ± 9.6 and 5.8 ± 7.0 mmHg/PU respectively), nor in the healthy subjects (2.2 ± 1.1 and 3.1 ± 3.3 mmHg/PU, respectively).

Correlations

There were no significant correlations between the differences in hormone levels before and after administration (i.e. the mean of the values at 90 and 180 min) and the differences in FST and LDF between the tests with placebo and with either sex hormone.

DISCUSSION

In the present study, short term administration of either 17ß-estradiol or progesterone has only minor influence on finger skin circulation both in healthy subjects and in Raynaud patients.

Some problems regarding the study design have to be considered. Intravenous administration of drugs should preferably be used to assess acute effects. Consequences of oral administration of sex steroids are the interindividual differences in absorption, the formation of metabolites in the gastrointestinal tract and the first pass effect in the liver. However, neither of the sex hormones is available in an intravenous form. For natural progesterone intramuscular administration is a parenteral alternative and for 17ß-estradiol chronic transdermal administration would be possible. Finally, to standardize the administration and to enable a double-blind design, we have chosen for the oral administration of both hormones.

Both sex hormones are rapidly metabolized. Metabolites of progesterone are 17α-hydroxyprogesterone and 20α-hydroxy-4 pregnene-3-one. The biological half-life of progesterone varies between 25 and 45 minutes and peak plasma levels can be expected after one to four hours after ingestion.[17,18] 17ß-Estradiol is metabolized (in the gut wall and in the liver) into estriol, estrone and estrone sulphate. The biological half-life of 17ß-estradiol is about one hour and peak plasma levels (after administration of 1.5 mg) are reached after one hour.[19,20] However, most of these metabolites are biologically less active or even inactive as compared with the precursor hormones and are not expected to influence our results in a major way. Because of the variability in absorption and the short biological half-life, the single-dose oral administration design results in variable high peak levels with subsequent rapid decrease of both hormones used. Nevertheless, we have reached sufficiently high levels of either hormone during the course of the whole test in almost all subjects, though the levels of 17ß-estradiol were more constant compared with the

levels of progesterone.

The acute administration used in this study is the next point of discussion. It is questionable whether an influence -if any- of 17ß-estradiol or progesterone can be detected immediately after a single oral dose of either hormone. It is possible that a more prolonged exposure to the sex hormones or a longer time period may be required to exert detectable effects.

Finally, more than the absolute concentration of one specific hormone, a certain relationship in concentrations of both sex hormones over time might be necessary to exert effects on the peripheral circulation. Besides the concentration of hormones per se, the ratio of 17ß-estradiol and progesterone or other sex hormones may play a major role. It could also be necessary that one hormone acts as the primer for the the other or that the rapid changes in hormone levels as seen during the menstrual cycle are of importance.

Up to now, no direct relationship between alterations in the peripheral circulation and plasma levels of estrogens or progestagens has been demonstrated in humans. Nevertheless, not only human arteries of the genital tract and the breasts, but also vascular smooth muscle of the heart, aorta and other large arteries have been shown to contain estrogen[21] and progesterone[22] receptors. The presence of these receptors may influence vessel wall reactivity directly or modulate the action of various vasoactive factors (e.g. catecholamines, angiotensin, acetylcholine, histamine, prostaglandins). Besides an influence of sex hormones on vascular tone, hemorheological variables, like viscosity and erythrocyte deformability that have an important influence on the microcirculation, showed a correlation with estradiol levels during the menstrual cycle.[23]

Animal studies did show an influence of both natural and synthetic estrogens and progestagens on different vascular beds.[1] Acute or chronic administration of natural or synthetic estrogens increases vascular responsiveness to adrenergic agonists in some vascular beds. Whether this also occurs in humans, remains unclear. On the contrary, our study suggests a skin vasodilatory effect of 17ß-estradiol intake, though the changes did not reach significance.

Effects of exogenous sex hormones and of the cyclical variations in endogenous hormones have been investigated on human platelet adrenoceptors, with conflicting results.[24,25] The influence of estrogens and progestagens on peripheral vascular human adrenoceptors has not been investigated yet. Finger blood flow responses to brachial artery infusions of adrenergic agonists differed between men and women, indicating that the sensitivity and/or density of peripheral vascular adrenoceptors is lower in women.[26]

In the literature, the effect of either 17ß-estradiol or progesterone on blood pressure

is not straightforward. During the menstrual cycle morning blood pressure has been shown to vary significantly, with the highest values during menstruation and the lowest in the luteal phase.[27] Natural progesterone (in contrast with synthetic progesterone) exerts a blood pressure lowering effect through an anti-mineralocorticoid action at doses which result in plasma levels that are just above luteal phase concentrations.[28] It is unlikely that the short term elevation of progesterone levels would already induce a drop in blood pressure in our normotensive subjects. Indeed, no significant differences between the placebo and the progesterone test in either systolic, diastolic or mean arterial blood pressure were found. Effects on blood pressure of estrogens are attributed to retention of salt and water by an effect on the renin-angiotensin system. However, no changes in blood pressure were described after either chronic oral conjugated estradiol or percutaneous 17ß-estradiol administration, although plasma renin substrate increased in the group using oral estradiol.[29] Again, these influences are present during chronic administration and hence can not be expected just after short term administration. Surprisingly, a decrease in diastolic and mean arterial blood pressure and an increase in heart rate were found after 17ß-estradiol administration in our study, although only in the Raynaud patients. In contrast, FBF and FVR as well as laser Doppler resistance in the skin did not change significantly in this group.

In conclusion, no major influence of either 17ß-estradiol or progesterone on finger skin circulation could be demonstrated after single-dose oral administration either in healthy female volunteers nor in female patients with primary RP. Further studies are needed to investigate the effect of long-term administration and the effect of exposure to a combination of 17ß-estradiol and progesterone. In this study no explanation could be found for the higher prevalence of RP in females and for the hormonal influence on the complaints of Raynaud patients.

REFERENCES

1 Altura BM, Altura BT. Influence of sex hormones, oral contraceptives and pregnancy on vascular muscle and its reactivity. In: Carrier O, Shibata S, eds. Factors influencing vascular reactivity. Tokyo: Igaku-Shoin 1977:221-54.
2 Cooke JP, Creager MA, Osmundson PJ, Shepherd JT. Sex differences in the control of cutaneous blood flow. Circulation 1990;82:1607-15.
3 Van de Wal HJCM, Wijn PFF, van Lier HJJ, Kneepkens WGHJ, Skotnicki SH. Noninvasive hemodynamic assessment of vasospasm in patients with primary Raynaud's phenomenon. Angiology 1987;38:315-32.
4 Bartelink ML, Wollersheim H, Theeuwes A, van Duren D, Thien Th. Changes in skin blood flow during the menstrual cycle: The influence of the menstrual cycle on the peripheral circulation in healthy female volunteers. Clin Sci 1990;78:527-32.

5 Tooke JE, Tindall H, McNicol GP. The influence of a combined oral contraceptive pill and menstrual cycle phase on digital microvascular haemodynamics. Clin Sci 1981;61:91-5.
6 Katz M, Sokal MM. Skin perfusion in pregnancy. Am J Obstet Gynecol 1980;137:30-3.
7 Gifford RW, Hines EA. Raynaud's disease among women and girls. Circulation 1957,16:1012-21.
8 Wollersheim H, Thien Th. The diagnostic value of clinical signs and symptoms in patients with Raynaud's phenomenon. A cross-sectional study. Neth J Med 1990;37:171-82.
9 Allen EV, Brown GE: Raynaud's disease, a critical review of minimal requisites for diagnosis. Am J Med Sci 1932;183:187-200.
10 Wollersheim H, Thien Th, Hoet MH, van Venrooy WJ. The diagnostic value of several immunological tests for anti-nuclear antibody in predicting the development of connective tissue disease in patients presenting with Raynaud's phenomenon. Eur J Clin Invest 1989;19:535-41.
11 Cleophas TJM, Fennis JFM, van 't Laar A. Finger temperature after a finger cooling test. Influence of air temperature and cigarette smoking. J Appl Physiol 1982;52:1167-71.
12 Thomas CMG, Corbey RS, Rolland R. Assessment of unconjugated oestradiol and progesterone levels throughout pregnancy in normal women and in women with hyperprolactinaemia who conceived after bromocriptine treatment. Acta Endocrinol 1977;86:405-14.
13 Kremer JAM, Borm G, Schellekens LA, Thomas CMG, Rolland R. Pulsatile secretion of luteinizing hormone and prolactin in lactating and nonlactating women and the response to naltrexone. J Clin Endocrinol Metab 1991;72:294-300.
14 Durnin JVGA, Womersley J. Body fat assessed from total body density and its estimation from skinfold thickness. Br J Nutr 1974;32:77-97.
15 Lenders J, Janssen GJ, Smits P, Thien Th. Role of the wrist cuff in forearm plethysmography. Clin Sci 1991;80:413-7.
16 Jansen TLThA, Tan ACITL, Wollersheim H, Benraad ThJ, Thien Th. Age-dependent vasodilation of the skin microcirculation by atrial natriuretic factor. J Cardiovasc Pharmacol 1991;18:622-30.
17 Sitruk-Ware R, Bricaire C, de Lignieres B, Yaneva H, Mauvais-Jarvis P. Oral micronized progesterone. Contraception 1987;36:373-402.
18 Whitehead MI, Townsend PT, Gill DK, Collins WP, Campbell S. Absorption and metabolism of oral progesterone. Br Med J 1980;280:825-7.
19 Longcope C, Gorbach S, Goldin B, Woods M, Dwyer J, Warram J. The meatabolism of estradiol; oral compared to intravenous administration. J Steroid Biochem 1985;23:1065-70.
20 Dada OA, Laumas V, Landgren BM, Cekan SZ, Diczfalusy E. Effect of graded oral doses of oestradiol on circulating hormonal levels. Acta Endocrinol 1978;88:754-67.
21 Campisi D, Bivona A, Paterna S, Valenza M, Albiero R. Oestrogen binding sites in fresh human aortic tissue. Int J Tiss Reac 1987;9:393-8.
22 Ingegno MD, Money SR, Thelmo W, Greene GL, Davidian M, Jaffe B, Pertschuk LP. Progesterone receptors in the human heart and the great vessels. Lab Invest 1988;59:353-6.
23 Solerte SB, Fioravanti M, Spinillo A, Ferrari E, Guaschino S. Association between hormonal and haemorheological changes during the menstrual cycle in healthy women. Br J Obstet Gynaecol 1988;95:1305-8.
24 Jones SB, Bylund DB, Rieser CA, Shekim WO, Beyer JA, Carr GW. α_2-Adrenergic receptor binding in human platelets: Alterations during the menstrual cycle. Clin Pharmacol Ther 1983;34:90-6.
25 Sundaresan PR, Madan MK, Kelvie SL, Weintraub M. Platelet $\alpha_2$adrenoceptors and the menstrual cycle. Clin Pharmacol Ther 1985;37:337-42.

26 Freedman RR, Sabharwal SC, Desai N. Sex differences in peripheral vascular adrenergic receptors. Circ Res 1987;61:581-5.
27 Dunne FP, Barry DG, Ferriss JB, Grealy G, Murphy D. Changes in blood pressure during the normal menstrual cycle. Clin Sci 1991;81:515-8.
28 Rylance PB, Brincat M, Lafferty K, de Trafford JC, Brincat S, Parsons V, Studd JWW. Natural progesterone and antihypertensive action. Br Med J 1985;290:13-4.
29 Hassager C, Riis BJ, Strφm V, Guyene TT, Christiansen C. The long-term effect of oral and percutaneous estradiol on plasma renin substrate and blood pressure. Circulation 1987;76:753-8.

CHAPTER 11

SUMMARY AND CONCLUSIONS

In this thesis we presented several epidemiological, methodological and clinical studies on vasospastic syndromes. As outlined in the general introduction, we tried to find answers to the following questions:

1. What is the prevalence in males and females of both migraine and Raynaud's phenomenon (RP)? Do these disorders often occur simultaneously?
2. Do female sex hormones have an effect on these vasospastic complaints? Do sex hormones influence the regulation of peripheral blood flow? And if so, which of the sex hormones could be responsible?

The first part of this thesis deals with epidemiological aspects of RP and migraine.

For migraine (Chapter 2) the average annual prevalence was 4 per 1000 men and 16 per 1000 women. Of 140 patients diagnosed as having migraine by their general practitioners 85% fulfilled the criteria of the International Classification of Health Problems in Primary Care. In this group the female to male ratio was 5:1. Despite substantial overlap migraine differed from non-vasospastic tension headache regarding photo- and phonophobia, duration and provoking factors. RP was present in 15% of the migraine patients (n=102) but also in 16% of the patients with tension headaches (n=56), almost exclusively women. Especially the complaints of migraine were perceived to increase during and before menstruation and to decrease during pregnancy and menopause.

To assess the prevalence of RP (Chapter 3) 508 subjects (202 men and 306 women) visiting their general practitioners completed a questionnaire. When strict criteria (cold digits, numbness and at least a biphasic discoloration) were applied, prevalence was 0.5% in males and 2.9% in females. When monophasic discolorations were included, prevalence increased largely (by 5.4% and 7.5%, respectively). Complaints of cold digits were present in 22.7% of the males and in 35% of the females. Therefore we conclude that the variation in prevalence of RP, as reported in the literature, can be explained to a large extent by differences in definition.

At the out-patient clinic a detailed questionnaire was completed by 130 primary Raynaud patients (31 males, 99 females), whereas (for the duration of three months) 27 females kept a diary with daily registration of frequency, severity and duration of the vasospastic attacks (Chapter 4). From the results of the questionnaire it turned out that complaints decreased during pregnancy in 6 out of 23 females. No influence of the menopause or the use of oral contraceptives was found. In some phase of the menstrual cycle an exacerbation was reported by 15 out of 80 females. The diaries, however, did not support the existence of influence of the menstrual cycle phase. Migraine was present in 21% of the Raynaud patients. We conclude that in contrast to most reports in literature and contrary to the results of laboratory research, most females do not experience an important influence of their sex hormonal status on the frequency, duration and severity of their vasospastic attacks.

The second part of this thesis comprises studies on the finger skin blood flow. We first discuss the finger cooling test we have used in these studies. During standardized cold challenge the finger skin temperature and the laser Doppler flux were measured. As described in Chapter 5, we did not find any relation between subjective complaints of RP, recorded in a diary, and the objective measurement of the finger blood flow during cooling. In contrast to findings obtained in a previous study in a small selected group, the reproducibility of the finger cooling test appeared to be only moderate. Laser Doppler readings showed a high variability and a poor reproducibility when compared with temperature measurements, particularly in the group of healthy subjects (Chapter 6). There were important differences in response to cold provocation between males and females both in healthy subjects and in Raynaud patients (Chapter 7): the skin blood flow in females was much lower. Although the finger skin temperature and the laser Doppler flux are significantly higher in the group of healthy subjects compared with the group of Raynaud patients, the diagnostic value of the finger cooling test in individual cases is limited: when a specificity of 70% was assumed a sensitivity of between 55% and 81% for the different parameters was obtained.

In Chapter 8 we show that, although we did not find an influence of the menstrual cycle on complaints of RP (see Chapter 4), the finger blood flow varies significantly within one menstrual cycle. There was a poor recovery after cooling in the mid-luteal phase (with high endogenous levels of both 17ß-estradiol and progesterone) compared with the best recovery in the pre-ovulatory phase (with high levels of 17ß-estradiol and low levels of progesterone). Within subjects we found a positive correlation between the 17ß-estradiol/progesterone ratio and the mean finger skin temperature during recovery, suggesting that besides the absolute concentrations of the different sex hormones their ratio may be important.

In Chapter 9 we compare males and females with different hormonal status using the finger heating and cooling test. Women using oral contraceptives and premenopausal women showed the most pronounced vasodilation during heating as well as the most pronounced vasoconstriction during cooling, while in the recovery period after cooling vasoconstriction endured. Males and postmenopausal women showed less vasodilation during heating, less vasoconstriction during cooling and recovered more rapidly after cooling. We concluded that vascular reactivity towards both heat and cold challenge seems to be enhanced by both synthetic and natural estrogens and progestagens. The findings in the postmenopausal and male group could be ascribed to the absence of female sex hormones or, alternatively, to the presence of androgens in (relatively) high concentrations. However, rather than the concentration of a specific sex hormone the ratio between 17ß-estradiol, progesterone or testosterone may be critical.

In the placebo controlled and double blind study in Chapter 10 no evidence is

found for the influence of either 17ß-estradiol or progesterone on the finger skin circulation after single-dose oral administration, both in healthy subjects and in Raynaud patients. There were no significant relations between hormone levels and differences in finger skin temperature and laser Doppler flux. Further studies are needed to investigate the effects of chronic exposure to a single hormone and of combined exposure to 17ß-estradiol and progesterone.

In conclusion, prevalence of both migraine and RP differs between males and females. For women with migraine the relationship between their migraine and their hormonal status is much more apparent than for women with non-vasospastic tension headache. In RP this relationship is less clear. Measurement of the finger skin blood flow clearly shows sex differences and an influence of the hormonal status in females. However, single-dose administration of the female sex hormones exerts no relevant influence on the finger skin perfusion. Although it still seems likely that sex hormones may play an important role, we cannot attribute the observed sex differences and the variation in skin blood flow during life to any specific sex hormone.

HOOFDSTUK 12

SAMENVATTING

INLEIDING

Vasospasme (vaatwandspasme)

Vasospasme veroorzaakt de aanvallen van witte, blauwe en rode verkleuringen van de vingers of de tenen, onder invloed van koude of stress, bij het fenomeen van Raynaud ('dode vingers'). Ook bij migraine speelt vasospasme een belangrijke rol, of als primaire oorzaak of secundair aan veranderingen in de hersenen. Verder is bekend dat vasospasme ook voorkomt in bloedvaten van andere organen, zoals het hart en de longen.

Een patiënt kan last hebben van zowel het fenomeen van Raynaud als van migraine. Er zijn dan ook aanwijzingen dat een gemeenschappelijke factor verantwoordelijk zou kunnen zijn voor het optreden van vasospasme in meerdere vaatgebieden.

Epidemiologie

De diagnose 'fenomeen van Raynaud' wordt zelden opgenomen in registraties in de huisartspraktijk. Dit geeft aan dat de huisarts de klachten van het fenomeen van Raynaud niet onderkent of dat de verschijnselen ervan niet vaak worden gepresenteerd. Inderdaad blijkt uit studies dat voor de meeste mensen de klachten niet ernstig of alarmerend genoeg zijn om een arts te consulteren. De in bevolkingsonderzoeken gerapporteerde prevalentie (het vóórkomen) varieert echter van 5% tot 20%, afhankelijk van de gebruikte definitie, het lokale klimaat en de samenstelling (bijvoorbeeld wat betreft geslacht of leeftijd) van de onderzochte groep. Voor veel mensen zijn de klachten van het fenomeen van Raynaud zeer onaangenaam in het dagelijkse leven of bij het werken in een koude omgeving, zeker in het koude en natte Westeuropese klimaat.

De prevalentie van migraine ligt tussen de 2% en 25%. De verschillen zijn te wijten aan de geslachtsverdeling en de leeftijdsopbouw van de onderzochte groep, en de gebruikte criteria om de diagnose te stellen. Vaak is het moeilijk om migraine te onderscheiden van andere soorten hoofdpijn, te meer daar er waarschijnlijk overgangen bestaan: van spanningshoofdpijn via migraine zonder naar migraine met neurologische verschijnselen. Hoewel migraine niet zo vaak voorkomt als andere vormen van hoofdpijn, is het vanwege de ernst van de klachten voor patiënten een belangrijk probleem.

Invloed van vrouwelijke geslachtshormonen

In de literatuur wordt vermeld dat het fenomeen van Raynaud tussen de 2 en 9 maal

vaker voorkomt bij vrouwen. Ook uit experimentele studies blijken sekse-verschillen: de gemeten vingerhuiddoorbloeding bij premenopauzale vrouwen is tot de helft lager dan bij mannen en bij vrouwen na de menopauze. Ook is beschreven dat er variaties in vingerhuiddoorbloeding optreden tijdens de menstruele cyclus. In deze laatste studies werden echter geen eenduidige veranderingen gevonden in een bepaalde fase van de cyclus en tevens ontbraken correlaties met de circulerende vrouwelijke geslachtshormonen 17ß-oestradiol en progesteron. In een enkel geval is beschreven dat tijdens zwangerschap de klachten verminderen. Het gebruik van de pil zou de subjectieve klachten doen verergeren, hoewel in een experimentele studie de gemeten vingerhuiddoorbloeding juist toenam.

Interessant is het dat ook migraine vaker voorkomt bij vrouwen. Men neemt aan dat er een relatie met de menstruele cyclus is, hoewel ook hier geen duidelijke fase aangewezen kan worden waarin de klachten bij iedereen verergeren. Een verergering, maar ook een vermindering van de hoofdpijnaanvallen tijdens pilgebruik, tijdens zwangerschap en na de menopauze is beschreven.

Al deze bevindingen wijzen in de richting van een invloed van geslachtshormonen op het ontstaan van vasospasme. Helaas zijn er geen studies waarin een relatie wordt gevonden tussen de normaal aanwezige geslachtshormonen en veranderingen in de vingerhuiddoorbloeding of het optreden van migraine-aanvallen. Bovendien maken de uiteenlopende resultaten van de eerder genoemde studies het onmogelijk een bepaald hormoon aan te wijzen dat verantwoordelijk zou zijn voor het optreden van vasospasme. Kortom, er bestaat nog veel onduidelijkheid. In dierstudies is wel een invloed aangetoond van zowel natuurlijke als synthetische oestrogenen, progestagenen en androgenen op verschillende vaatgebieden.

De verandering in de doorbloeding van de vingerhuid als reactie op koude wordt geregeld door het sympathisch zenuwstelsel. De huidbloedvaten zijn zodoende belangrijk bij de temperatuurregulatie. Bij stimulatie van het sympathisch zenuwstelsel door koude, maar ook bij stress, komen catecholamines (adrenaline en noradrenaline) vrij die verschillende receptoren in de vaatwand stimuleren, zodat vasoconstrictie (vaatvernauwing) of vasodilatatie (vaatverwijding) optreedt in de huidvaten. Een geringe stoornis ergens in dit complexe systeem zou al tot een aanzienlijk vasospasme kunnen leiden, zoals door Maurice Raynaud al in 1862 werd verondersteld. Er zijn onderzoeken die wijzen op een invloed van oestrogenen op het sympathisch zenuwstelsel in die zin dat oestrogenen de hoeveelheid vasoconstrictieve α_2-receptoren in de bloedvatwand zouden doen toenemen. Ook bij het ontstaan van migraine wordt een rol toegekend aan het sympathisch zenuwstelsel, dat op zijn beurt weer beïnvloed zou kunnen worden door geslachtshormonen. Wellicht kunnen geslachtshormonen vasospasme induceren via beïnvloeding van het sympathisch zenuwstelsel.

Meettechnieken en provocatietesten

Om vasospasme te objectiveren dient de vingerhuiddoorbloeding gemeten te worden. Het kwantificeren van de perifere circulatie is echter moeilijk, omdat onder normale omstandigheden de huiddoorbloeding aan sterk varieert, met grote veranderingen in een zeer korte periode onder invloed van diverse stimuli. Er worden verschillende koude-provocatietesten gebruikt om vasospasme te bestuderen. Sommige onderzoekers maken gebruik van lokale koeling, anderen koelen de ene en meten aan de andere hand en weer anderen koelen het gehele lichaam. Evenzo wordt een heel scala aan meettechnieken gebruikt. Er is daarom weinig uniformiteit in deze testen. Ze worden meestal niet voldoende gestandariseerd uitgevoerd, en de reproduceerbaarheid noch de diagnostische waarde ervan zijn vastgesteld.

Samenvattend werd geprobeerd een antwoord te vinden op de volgende vragen:
1. Wat is de prevalentie van migraine en het fenomeen van Raynaud bij mannen en bij vrouwen. Komen deze aandoeningen vaak gecombineerd voor?
2. Hebben vrouwelijke geslachtshormonen een invloed op de klachten van patiënten met deze vasospastische aandoeningen? Beïnvloeden vrouwelijke geslachtshormonen de perifere circulatie? Welke van de geslachtshormonen is in dat geval verantwoordelijk?

INHOUD VAN DIT PROEFSCHRIFT

In het eerste deel worden epidemiologische aspecten van migraine en het fenomeen van Raynaud bestudeerd.

Om de invloed van vrouwelijke geslachtshormonen te onderzoeken, zijn de prevalentie bij mannen en vrouwen en de invloed van respectievelijk menarche, menopauze, zwangerschap, pilgebruik en de fase van de menstruele cyclus bestudeerd bij patiënten met migraine of het fenomeen van Raynaud.

Hoofdstuk 2 beschrijft de prevalentie van migraine in de huisartspraktijk, zoals die volgens specifieke criteria geregistreerd wordt in de Continue Morbiditeitsregistratie (de CMR). Verder is gekeken naar het tegelijkertijd voorkomen van het fenomeen van Raynaud en andere vasospastische aandoeningen en naar de samenhang met stress en vrouwelijke geslachtshormonen. Een controlegroep met (niet-vasospastische) spanningshoofdpijn werd geselecteerd en vergeleken met de migrainegroep.

In Hoofdstuk 3 wordt de prevalentie van het fenomeen van Raynaud beschreven en de invloed van verschillende criteria die gebruikt worden om deze diagnose te stellen. Omdat deze diagnose niet was opgenomen in de CMR, is een grote groep patiënten uit verschillende huisartspraktijken ondervraagd.

In Hoofdstuk 4 wordt de invloed van vrouwelijke geslachtshormonen op de

subjectieve klachten van het fenomeen van Raynaud onderzocht.

Het tweede gedeelte van dit proefschrift bevat studies over de gemeten vingerhuiddoorbloeding. We gebruikten een gestandariseerde vingerkoelingstest (5 minuten lokale afkoeling in een waterbad van 16°C, waarna een herstelperiode van 20 minuten) met registratie van de vingerhuidtemperatuur en de laser Doppler flux. Deze laatste meetmethode maakt gebruik van laserlicht dat op de vingerhuid valt. Dit licht ondergaat door de stromende rode bloedcellen een frequentie-verschuiving (Doppler-effect). Een deel van dit teruggekaatste licht wordt weer opgevangen, en via het Doppler-principe omgerekend in 'rode-bloedcelsnelheden': een maat voor de bloeddoorstroming.

In Hoofdstuk 5 wordt de relatie tussen de resultaten van deze test en de subjectieve ernst van de klachten van patiënten met het fenomeen van Raynaud bestudeerd. De reproduceerbaarheid van de test bij een groep van 90 personen wordt beschreven in Hoofdstuk 6. In Hoofdstuk 7 worden de diagnostische waarde van deze test en de verschillen in testresultaten tussen mannen en vrouwen beschreven.

Om de cyclische invloed van vrouwelijke geslachtshormonen te bestuderen, werd de vingerhuiddoorbloeding bestudeerd in verschillende fasen van de menstruele cyclus bij gezonde vrijwilligsters (Hoofdstuk 8).

Om de test beter te standariseren (er werd gestart met een waterbad van 30°C, waardoor de uitgangswaarde van de vingertemperatuur zoveel mogelijk gelijk gemaakt werd) en om ook een warmteprovocatie toe te voegen (10 minuten in water van 45°C), werd een gecombineerde vingerverwarmings- en vingerkoelingstest uitgevoerd. Met deze test werden verschillen in vingerhuiddoorbloeding tussen groepen proefpersonen met een verschil in hormonale status bestudeerd: mannen en drie groepen vrouwen: pilgebruiksters, premenopauzale en postmenopauzale vrouwen (Hoofdstuk 9).

Tot slot onderzochten we in een dubbel-blinde, placebo-gecontroleerde studie de invloed van eenmalige toediening van de natuurlijke vrouwelijke geslachtshormonen 17ß-oestradiol en progesteron op de perifere circulatie bij gezonde vrijwilligsters en bij patiënten met een primair fenomeen van Raynaud (Hoofdstuk 10).

RESULTATEN EN CONCLUSIES

De jaarlijkse prevalentie van migraine in de CMR was 4 per 1000 mannen en 16 per 1000 vrouwen (Hoofdstuk 2). Migraine verschilde wat betreft de duur van de hoofdpijn, de begeleidende verschijnselen, provocerende factoren en medicijngebruik duidelijk van (niet-vasospastische) spanningshoofdpijn. Het fenomeen van Raynaud was aanwezig bij 15% van de migraine-patiënten en bij 16% van de groep met spanningshoofdpijn, bijna uitsluitend bij vrouwen. Vooral migraine verergerde tijdens

en kort voor de menstruatie en verbeterde tijdens zwangerschap en de menopauze.

Om de prevalentie van het fenomeen van Raynaud te bepalen vulden 508 patiënten die hun huisarts bezochten een vragenlijst in. Indien 'strenge' criteria werden toegepast (koude vingers, doof gevoel en een bi- of trifasische verkleuring) was de prevalentie 0,5% bij mannen en 2,9% bij vrouwen. Als ook monofasische verkleuringen werden meegerekend, steeg de prevalentie tot 22,7% bij mannen en 35,0% bij vrouwen. Daarom wordt in Hoofdstuk 3 geconcludeerd dat een groot gedeelte van de variatie in prevalentiecijfers in de literatuur berust op de verschillen in de gebruikte definities.

Onder de patiënten met het fenomeen van Raynaud die gecontroleerd worden op de polikliniek Inwendige Geneeskunde van het Academisch Ziekenhuis Nijmegen werd een enquête gehouden, om de invloed van de hormonale status op de klachten te onderzoeken. Een aantal van deze vrouwen noteerde gedurende drie maanden dagelijks in een dagboek de ernst, de frequentie en de duur van de verkleuringen. Uit de enquête bleek dat de klachten in een aantal gevallen tijdens de zwangerschap verminderden. Er werd geen invloed gevonden van de menopauze of van pilgebruik. Een verergering van de klachten in een bepaalde fase van de menstruele cyclus werd gevonden bij enkele van de vrouwen in de enquête, maar deze bevinding werd niet gesteund door de resultaten van het dagboekonderzoek. Migraine kwam voor bij 21% van deze Raynaud-patiënten. We concluderen derhalve, dat vrouwen in het dagelijkse leven geen belangrijke invloed ervaren van vrouwelijke geslachtshormonen op de klachten van het fenomeen van Raynaud (Hoofdstuk 4). Dit is in tegenstelling met wat in de literatuur beweerd wordt en in tegenstelling met de resultaten van experimenteel circulatie-onderzoek.

In het tweede gedeelte van het proefschrift wordt eerst de gebruikte provocatietest besproken. In Hoofdstuk 5 wordt beschreven dat er geen relatie is tussen de subjectieve klachten van patiënten en de meetresultaten. In tegenstelling tot een eerdere studie, uitgevoerd in een kleine geselecteerde groep, bleek de reproduceerbaarheid van de vingerkoelingstest matig. Vooral de laser Doppler metingen vertoonden grote variatie en de reproduceerbaarheid ervan was slechter dan die van de temperatuurmetingen, vooral in de groep van de gezonde proefpersonen (Hoofdstuk 6). Vrouwen hadden een lagere vingerhuiddoorbloeding dan mannen. Hoewel de vingerhuiddoorbloeding bij patiënten met een fenomeen van Raynaud lager was dan bij gezonde vrijwilligers, bleek de diagnostische waarde van deze test in individuele patiënten matig, zowel vanwege de matige specificiteit (percentage terecht als gezond geclassificeerden) als vanwege de matige sensitiviteit (percentage terecht als Raynaud-patiënt geclassificeerden).

In Hoofdstuk 8 wordt aangetoond dat, ondanks het ontbreken van een invloed van de menstruele cyclus op de klachten, de gemeten vingerhuiddoorbloeding een duidelijk

cyclische variatie vertoont. In de luteale fase (hoge concentraties van oestradiol en progesteron) vertoonden de vrouwen een slechter herstel na koelen in vergelijking met het beste herstel na koelen in de pre-ovulatoire fase (hoge concentratie oestradiol, lage concentratie progesteron). Een positieve correlatie werd gevonden tussen de oestradiol/progesteron-ratio en de resultaten in de herstelperiode. Dit suggereert dat de verhouding tussen de verschillende geslachtshormonen belangrijker is dan de absolute concentraties van beide hormonen.

In Hoofdstuk 9 worden de resultaten van de verwarmings- en afkoelingstest van mannen en verschillende groepen vrouwen vergeleken. Pilgebruiksters en premenopauzale vrouwen lieten een sterkere vasodilatatie na verwarmen en een sterkere vasoconstrictie na afkoelen zien dan mannen en postmenopauzale vrouwen. We concludeerden dat de vasculaire reactiviteit na verwarmen en afkoelen verhoogd lijkt in de aanwezigheid van zowel synthetische als natuurlijke vrouwelijke geslachtshormonen. Een verklaring voor de resultaten bij mannen en postmenopauzale vrouwen zou de lage concentratie vrouwelijke geslachtshormonen, dan wel de relatief hoge concentratie mannelijke geslachtshormonen kunnen zijn. Ook hier lijkt voor de hand te liggen dat niet de absolute hoeveelheid van een bepaald geslachtshormoon, maar de onderlinge verhouding het belangrijkst is.

In de dubbel-blinde, placebo-gecontroleerde laatste studie (Hoofdstuk 10) werd geen duidelijke invloed van eenmalig toegediend oestradiol en progesteron op de vingerhuiddoorbloeding gevonden. Er waren geen significante correlaties tussen de hormoonspiegels en de testresultaten. Er zijn meer studies nodig om de effecten van chronische of van gecombineerde toediening van geslachtshormonen te bestuderen.

We vinden duidelijke verschillen tussen mannen en vrouwen in de prevalentie van zowel migraine als van het fenomeen van Raynaud. Beide aandoeningen komen inderdaad vaak samen voor.

Vrouwen met migraine kunnen beter dan vrouwen met spanningshoofdpijn een verband tussen de hormonale status en hun klachten aangeven; bij vrouwen met het fenomeen van Raynaud is dit verband niet zo duidelijk. Meting van de vingerhuiddoorbloeding laat een duidelijke invloed van sekse en hormonale status zien. Desondanks heeft eenmalige toediening van 17ß-oestradiol en van progesteron geen invloed op de vingerhuiddoorbloeding. Hoewel het duidelijk lijkt dat geslachtshormonen de vaatwandreactiviteit beïnvloeden, kunnen de gevonden sekseverschillen nog niet goed verklaard worden en kan de variatie in verschijnselen en de gemeten vingerhuiddoorbloeding bij verschillende hormoonspiegels nog niet aan een specifiek hormoon toegeschreven worden.

DANKWOORD

In het kort wil ik enkele mensen hartelijk danken die meegewerkt hebben aan het tot stand komen van dit proefschrift. Allereerst ben ik de patiënten met het fenomeen van Raynaud en met migraine en de vele andere proefpersonen die hebben meegewerkt aan het onderzoek veel dank verschuldigd. Verder dank ik de leden van de projectgroep: Hub Wollersheim, Theo Thien, Eloy van de Lisdonk en Chris van Weel; van hen heb ik het onderzoeken geleerd. Hans Vemer en Dyonne van Duren hebben me geadviseerd op gynaecologisch gebied, terwijl Chris Thomas en Rob van de Berg van het Gynaecologisch Laboratorium de hormoonbepalingen perfect verzorgden. Ad Theeuwes en Theo de Boo (MSA) en Henk van den Hoogen en Hans Bor (NUHI) stonden altijd klaar me te helpen met de statistische verwerking van de gegevens. De keuzevakstudenten Dorothé Panhuizen, Lian van Riet, Astrid de Wit, Rob Spruyt en Erik Leesmans hebben geholpen met het uitvoeren van gedeelten van het onderzoek. Ook wil ik de medewerkers (met name Joeke Reyenga, Eugénie Olde Riekerink en Joost den Arend) en medeonderzoekers van de 'circulatiegroep' bedanken, die me altijd hebben geholpen en gestimuleerd. Ten slotte bedank ik mijn ouders, Jeroen, en mijn vrienden voor de steun tijdens het voltooien van deze dissertatie.

CURRICULUM VITAE

Marie-Louise Bartelink werd op 21 maart 1961 geboren in Oldenzaal. Nadat zij aan het Canisisus College in Nijmegen in 1979 het VWO-diploma had gehaald, studeerde zij geneeskunde aan de Katholieke Universiteit Nijmegen. Eind 1987 deed zij haar artsexamen. Begin 1988 startte zij met het onderzoek (door de faculteit toegewezen in het kader van 'sekse-specifiek onderzoek geneeskunde') dat in dit proefschrift resulteerde. Het onderzoek werd uitgevoerd binnen de afdeling algemeen inwendige geneeskunde (hoofd destijds: Prof. Dr A. van 't Laar) en de vakgroep huisarts-geneeskunde (hoofd: Prof. Dr C. van Weel).

ISBN 90-9005580-0